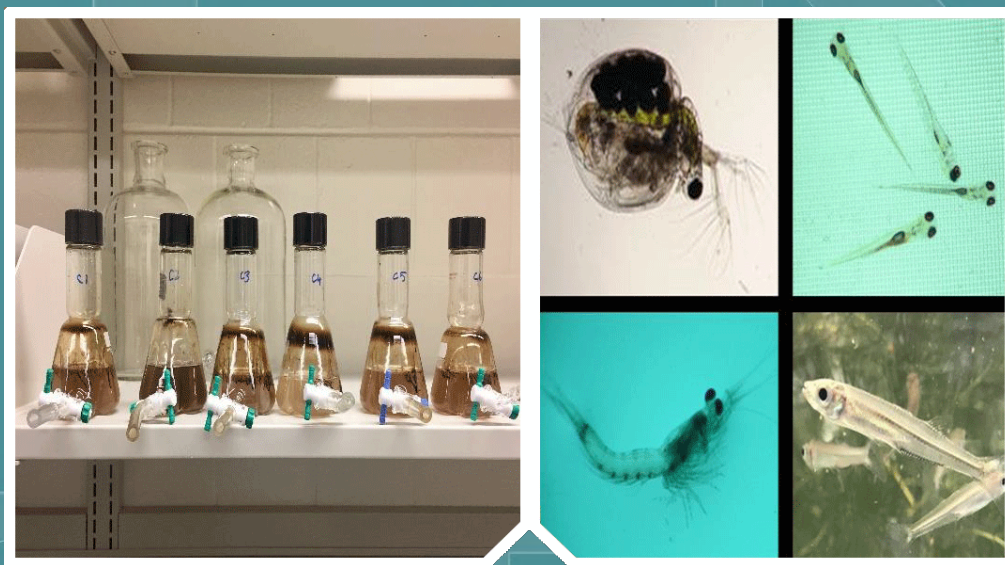# Characterization and Behavior of Cold Lake Blend and Western Canadian Select Diluted Bitumen Products

Characterization and Behavior of Cold Lake Blend and Western Canadian Select Diluted Bitumen Products

by

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Foreword

The U.S. Environmental Protection Agency (US EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, US EPA's research programs are providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) within the Office of Research and Development (ORD) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

Cynthia Sonich-Mullin, Director
National Risk Management Research Laboratory

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Acronyms and Abbreviations

AFE	Anderson Ferry Enrichment
ANOVA	Analysis of Variance
ANS	Alaskan North Slope Crude Oil 521
API	American Petroleum Institute
Bbl	Billion Barrels
BFT	Baffled Flask Test
BTEX	Benzene-Toluene-Ethylbenzene-Xylene
Cryo	$5^{o}C$ microbial cultures
CLB	Cold Lake Blend
CHY	Chrysenes
DCM	Dichloromethane
DE	Dispersion Effectiveness
Dilbit	Diluted Bitumen
DOR	Dispersant-to-Oil Ratio
DSD	Droplet Size Distribution
EPA	Environmental Protection Agency
FID	Flame Ionization Detector
GC	Gas Chromatography
HMW	High Molecular Weight
IC25	25% Inhibition Concentration
KRE	Kalamazoo River Enrichment
LC20	Lethal Concentration to kill 20% of the population
LC50	Lethal Concentration to kill 50% of the population
LCL95	Lower Confidence Level 95 %
LDR	Linear Dynamic range
LISST	Laser In Situ Scattering and Transmissometry
Meso	$25^{o}C$ microbial cultures
MRM	Multi Reaction Monitoring
MSD	Mass selective Detector
NELAC	National Environmental Laboratory Accreditation Conference
NAP	Naphthalenes
NAS	National Academies of Science, Engineering & Medicine
NOEC	No Observable Effect Concentration
ORD	Office of Research and Development
PAH	Polycyclic Aromatic Hydrocarbons
PBC	Prudhoe Bay Crude
PHE	Phenanthrenes

PHMSA	Pipeline and Hazardous Materials Safety Administration
QAPP	Quality Assurance Project Plan
SARA	Saturates-Aromatics-Resins-Asphaltenes
SCO	Synthetic Crude Oil
SOP	Standard Operating Procedure
Synbit	Synthetic Bitumen
TPH	Total Petroleum Hydrocarbons
WAF	Water Accommodated Fraction
WCS	Western Canadian Select

Executive Summary

Unconventional diluted bitumen (dilbit) oil products present an increasing environmental concern because of extensive transport in North America, recent spills into aquatic habitats, and limited understanding of environmental fate and toxicity. Dilbits are blends of highly weathered bitumen and lighter diluent oils that contain higher concentrations of asphaltenes (>10%) and lower levels of saturates (~40%). They have unique properties, including high adhesion, and the potential for rapid weathering, sinking and associating with sediments. Information on dilbit biodegradation, toxicity, dispersion and fate is limited and warrants further study, particularly given diversity in dilbit types and weathering state. Recent reviews produced in collaboration by government agencies, the National Academies of Science, academics and industry highlight the pressing need to better understand the behavior and potential impacts of dilbit spills over land and water. To address knowledge gaps pertaining to the behavior, fate and effects of select fractions of spilled diluted bitumen, this report summarizes research conducted within the US EPA ORD on the chemical characterization, dispersion effectiveness, biodegradation and toxicity of two types of diluted bitumen- Western Canadian Select and Cold Lake Blend. These studies are also being published in Deshpande, 2016; Deshpande et al, 2017; Barron et al., 2017. The **objectives** of this research were to:

1. Characterize Cold Lake Blend (CLB) and Western Canadian Select (WCS) dilbit products (unweathered) via chemical and physical properties and dispersion effectiveness.
2. Evaluate the biodegradation of alkanes and aromatics within CLB and WSC dilbit products as function of two temperatures (5 and 25 $^\circ$C) using two freshwater cultures: one consortium acclimated to dilbit (Kalamazoo River Enrichment, KRE) and the other consortium enriched on soil contaminated with conventional hydrocarbons from the Ohio River (Anderson Ferry Enrichment, AFE). An analysis of microbial community structure via genomic sequencing was also conducted.
3. Determine the acute and sublethal toxicity of unweathered and weathered CLB and WCS dilbit to standard aquatic test organisms: the freshwater invertebrate *Ceriodaphnia dubia*, the freshwater fish *Pimephales promelas* (fathead minnow), the saltwater invertebrate *Americamysis bahia* (mysid), and the saltwater fish *Menidia beryllina* (inland silverside). Conventional slow-stir water accommodated fractions (WAF) and static or static renewal methods were used to allow comparison to the broader literature on the toxicity of oil products.

This research will allow for better informed risk assessments and improved emergency response during dilbit spills in aquatic and terrestrial environments.

Results of our study suggest that select fractions of Western Canadian Select (WCS) and Cold Lake Blend (CLB) dilbit can be efficiently biodegraded, but under similar conditions, conventional crude oil (Prudhoe Bay Crude; PBC) was eliminated more effectively due to the higher content of lighter hydrocarbons. The rates of alkane and polycyclic aromatic hydrocarbons (PAH) degradation were comparable between the two dilbit products, but the extent of degradation was greater for conventional crude oil owing to the higher concentrations of lighter alkanes.

The potential of microbial enrichment to degrade crude oil was highly influenced by temperature as well as the composition. Lower degradation rates were achieved at the lower temperature, consistent with previous studies. Extended acclimation period and slower rates of degradation at lower temperatures may be due to lower solubilities and crystallization of hydrocarbons, and lower metabolic rates. As per the results of genomic sequencing, well-known oil degraders metabolized select fractions of both the dilbit types, but their performance varied. All the enrichments metabolized select fractions of the PBC as well WCS dilbit, but the nature and extent of the degradation was distinct. Kalamazoo River enrichment (KRE) meso culture was the most effective among all, as it completely removed alkanes and PAHs. Anderson Ferry enrichment (AFE) performed differently at two temperatures; where an acclimation period of 8 days was observed at 5 ºC while there was no lag at 25 °C. KRE meso culture as well as AFE culture at both the temperatures degraded alkanes completely while they were not able to metabolize heavier fractions of the oil (C_{2-4} homologues of 3 ring compounds and 4 ring compounds). Differences in the microbial activities can be explained by their composition, where the two cultures were a mixture of various microbial species and their composition was diverse. Diversity in the composition can be explained by difference their origin (Kalamazoo River vs. Ohio River) as well as the carbon source (dilbit vs conventional crude) on which they were enriched.

The aquatic effects were examined for select fractions of dilbit using four standard aquatic species. The results for these fractions indicate that dilbits can have similar acute and sublethal toxicity as crude oils and other petroleum products. Water accommodated fractions of the dilbits were characterized for total petroleum hydrocarbons (TPH), PAHs, and monoaromatics (benzene-toluene-ethylbenzene-xylene; BTEX). Acute toxicity of unweathered and weathered dilbits were similar for all four species ranging from 4 to 16 mg/L TPH, 8 to 40 ug/L total PAHs, and 0.7 to 16 mg/L BTEX. Weathered dilbits were sublethally toxic (impaired growth and reproduction) at 0.8 to 16 ug/L TPH and they can have similar acute and short term toxicity as other oils.

Results of this work serve to gain insight on the behavior, fate and potential hazards of select fractions of spilled diluted bitumen in the environment. Such research is needed to better prepare emergency responders for remediation options and to understand the persistence of dilbit in water. Increasing the knowledge base of dilbit has application in the risk assessments of

spills, and allows comparison of the relative hazards of dilbit products to conventional oil products. Further research is needed on other fractions of dilbit, especially the asphaltenes, as well as long term toxicity.

1. Introduction

Oil sands deposits consist of a mixture of oil, sand, clay and water (Yang et al., 2011). They are formed when petroleum reserves do not exceed 80 deg C, thus impeding pasteurization. Over geologic time, lighter, soluble petroleum compounds are evaporated and biodegraded as the oil cooled. This results in residual high molecular weight (HMW) oil compounds of high viscosity, sulfur and metal content, organic acids and asphaltenes (Lin et al., 1989; Crosby et al., 2013), termed bitumen. Bitumen is a heavy sour oil with a gas-free viscosity > 10,000 cSt (WEC, 2010) that can be either more or less than the density of pure water. Oil sands bitumen is found throughout the world, with the largest reservoir in Alberta, Canada. Alberta's reserves are estimated at 166 billion Barrels (bbl) and production of 2.3 million barrels per day (bbl/d) (Alberta Energy Regulator Agency, 2014), making it the third largest oil reserve in the world behind Venezuela and Saudi Arabia. The extraction of bitumen occurs via two ways. Surface mining is sufficient for deposits less than 75 m from the surface, where oil is separated from sand and water via centrifuge prior to preparation for transport. For deeper deposits, which is nearly 80% of the remaining reserves (Energy Information Administration, 2013; Canadian Energy Pipelines Association, 2013; Alberta Energy, 2016), *in situ* recovery is used. This unconventional technique is a method for using steam and solvent injection to separate and pump bitumen to the surface.

Due to the high viscosity of bitumen, it is difficult to transport through pipelines. The produced material does not meet transmission pipeline specifications, such as the Maximum Operating Pressure requirements administered by PHMSA (Pipeline and Hazardous Materials Safety Administration) in the U.S.A. Thus, for pipeline transport, bitumen must be diluted with diluent such as natural gas condensates, or lower density crude oils to form Diluted Bitumen, commonly known as *dilbit* (CAPP, 2012). Bitumen can also undergo an upgrading process to form a light, sweet 'synthetic' crude oil (SCO) that is then diluted to produce a synthetic bitumen (*synbit*). The mixture of bitumen and diluent varies but has a general target ratio of 70% bitumen to 30% diluent, allowing dilbits to be transported at comparable pipeline pressures as conventional heavy crudes (Canadian Government, 2013; Canadian Energy Pipelines Association, 2013; American Petroleum Institute, 2012). Table 1.1 shows how dilbits compare to heavy conventional crudes in North America. Although dilbits differ from conventional crude oils in adhesion properties and asphaltene / resin content, recent studies have shown that their corrosivity is akin to conventional crudes and considered to be low (Alberta Innovates, 2011; Penspen, 2013). Specific dilbit (Cold Lake Blend and Western Canadian Select) composition in terms of weight percent of saturates, aromatics, resins and, asphaltenes (SARA) as compared to other crudes is represented in Figure 1.1. Illustrated is that dilbit has higher concentrations of resins and asphaltenes, yielding it more viscous and denser as compared to other petroleum products. The average dilbit density ranges between 824 and 941 kg/m^3 at 15 °C with average API (American Petroleum Institute) gravity values between 18-39 (POLARIS, 2013).

Bitumen oil products are known as unconventional oils, in part because of extraction and transport preparation, but also because until recently they were not commonly extracted. In North America

bitumen products have been transported in small percentages for the past four decades (NAS, 2016). Improvements in extraction technologies, increased global demand and rising oil prices from the early 2000's led to an increase in dilbit production, (CAPP, 2015). Subsequently, shipment of dilbit via rail, pipeline and tanker has increased in North America over the last decade, where the U.S. imports 1.2 million barrels per day. Evidence of this can be gathered from mapping the existing and proposed dilbit pipelines, illustrated in Figure 1.2. The primary pipelines are TransCanada, Keystone, Enbridge and Kinder Morgan.

In the North America there have been three major dilbit spill incidents reported in the past 10 years. In 2007 a Trans Mountain Pipeline operated by Kinder Morgan Canada Inc. was punctured in Burnaby, British Columbia, Canada, resulting in a discharged volume of 224,000 liters of Albian Heavy (blend of synthetic crude oil and bitumen) that impacted soils, storm drains, sewer lines and 15 km of shoreline / marine waters of Burrard Inlet (Transportation Safety Board of Canada, 2007). The emergency response and remediation minimized the short and long-term effects of the spill, where approximately 218,000 liters of oil were recovered by skimming, booming and flushing of shorelines (Stantec Consulting Ltd., 2012). In July 2010, the Enbridge pipeline ruptured, releasing 3,320,000 liters of dilbit (Cold Lake and MacKay River Blends) into Talmadge Creek and Kalamazoo River, near Marshall, Michigan. Presence of floating, submerged and sunken oil was reported, and approximately 700,000 liters of oil still remains in the river submerged, bound to sediment for which dredging is being used (USEPA, 2013a). Most recently, a dilbit spill occurred in March 2013 near the suburban area of Mayflower, Arkansas. The ExxonMobil's Pegasus pipeline spill caused the release of 800,000 liters of Wabasca Heavy crude, which is a blend of bitumen and condensate (www.phmsa.dot.gov).
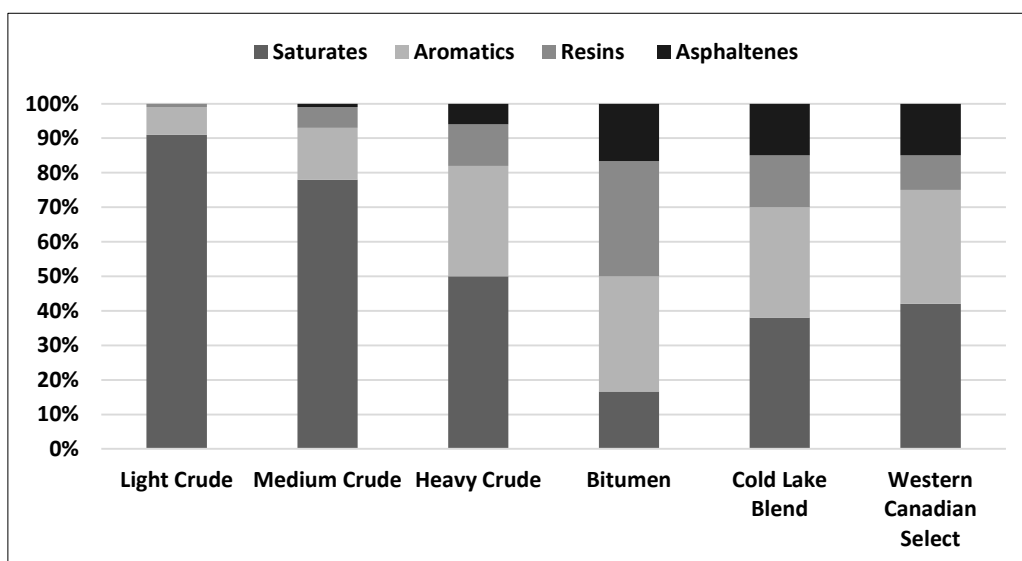
Figure 1.1. Saturate-Aromatic-Resin-Asphaltene (SARA) composition for different oils. Cold Lake Blend and Western Canadian Select are specific diluted bitumen products. Source: National Academies of Sciences, Engineering, and Medicine. Spills of Diluted Bitumen from Pipelines: A Comparative Study of Environmental Fate, Effects, and Response.

Table 1.1 Trace element concentrations in various heavy crude oil types. Source: American Petroleum Institute; 2011 White Paper; Pipeline transportation of diluted bitumen form the Canadian Oil Sands.

Location	Crude Name	API Gravity	Sulfur % wt	Vanadium (ppm)	Nickel (ppm)	Mercury (ppm)	Lead (ppm)
Canada	Bow River Heavy*	26.7	2.1	54	21	---	---
	Western Canadian Select[†]	20.6	3.4	134	56	---	---
	Cold Lake Blend**	22.6	3.6	169	65	---	---
	Wainwright-Kinsella **	23.1	1.6	80	40	---	---
California	California API 15	13.2	5.5	266	111	---	3
	California API 11	10.3	3.3	245	106	bdl	3
	Hondo	19.6	4.3	196	75	bdl	bdl
	Point Arguello Heavy	18.2	3.4	---	---	---	---
	Santa Clara	22.1	2.9	193	77	bdl	bdl
Iran	Soroosh	18.1	3.3	101	35	---	---
Mexico	Maya	21.3	3.0	257	44	bdl	bdl
Nigeria	Focardos Blend	29.7	0.3	---	---	---	---
Venezuela	Tia Juana Heavy	12.1	2.7	---	---	---	---
	Lago Treco	22.6	2.6	---	---	---	---
	Boscan	10.1	5.5	1320	117	bdl	bdl
	Bacaquero	16.8	2.4	---	---	---	---
	BCF 24	23.5	2.0	---	---	---	---

bdl = below detection limits; --- = no data reported

* = Conventional crude

** = Dil-bit

[†] = Made up of conventional and Dil-Bit streams as it is a special blend of various crude types

References:

Crude Monitor. 2011. Crudemonitor.ca. Website accessed 24 Jan 2011. Website: http://www.crudemonitor.ca/home.php.

Environment Canada. 2011. Oil Properties Database. Website accessed 24 Jan 2011. Website: http://www.etc-cte.ec.gc.ca/databases/oilproperties/.

Figure 1.2. Existing and proposed Canadian and U.S. oil pipelines for dilbit transport. *Source: Canadian Association of Petroleum Producers, also found at* https://www.nap.edu/read/21834/chapter/3#12*.*

Dilbits are a growing concern because of increasing transport in North America, recent high-profile spills with considerable impacts to communities, unique properties, and limited information on environmental fate and behavior in aquatic environments. Further, the composition of bitumen varies with the location of the deposits and since different diluents can be used for dilution purposes, there is no uniformity in the quality or the composition of dilbit. The specifications for diluents are vaguely stated in literature. It is suggested that diluents should have a density of 650-750 kg/m^3, maximum viscosity of 2 cSt, and no more than of 0.5 weight % of sulfur. The most frequently used diluents are condensates, which are liquid by-products of natural gas extraction processes containing mainly pentane and heavier hydrocarbons (Canadian Energy Pipelines Association, 2013; Crosby et al., 2013; Canadian Government, 2013). Thus hazard and risk assessment, and subsequent remediation options, remains complex because of the diversity of dilbit products that can vary geographically and seasonally in composition (Canadian Government, 2013; Polaris, 2013).

Dilbits can exhibit rapid environmental weathering with the loss of the diluent components (Canadian Government, 2013; Polaris, 2013). This equates to uncertainty in knowing if and over what timeframe they will sink or float as they weather. Recent studies have targeted the behavior of dilbit in water to better understand sinking and floating scenarios. In a 13-day weathering experiment within a flume tank, using filtered water, Access Western Blend was found to sink after 6 days, however Cold Lake Blend was more resistant to weathering due to higher concentration of alkylated polycyclic aromatic hydrocarbons (PAHs) and did not sink within 13 days (King et al., 2014). However, with unfiltered water and in the presence of particles, sinking could have occurred earlier. Weathered dilbit has also been shown to be

amenable to dispersion (Canadian Government, 2013) where dispersants were effective within the first 48 hours. Additionally, in situ burning can be considered within 96 hours of a spill, where the water is calm, the oil is greater than 2 mm thick, and the spill can be contained, as per the spill response options decision matrix presented in King et al., 2017. Although these studies are providing information on the behavior of dilbit, knowledge gaps still exist regarding the effects of suspended particles, salinity, or sunlight-induced oxidation on dilbit sinking.

Additionally, there are knowledge gaps with respect to dilbit biodegradation. Given that bitumen deposits are themselves residues remaining after extensive anaerobic biodegradation, evaporation and water washing of original crude oils (Lin et al., 1989; Yang et al., 2011; NAS, 2016), it has been posited that a spill of diluted bitumen may be less susceptible to biodegradation than a comparable spill of light or medium crude oil. This is supported by the presence of large unresolved complex mixtures in chromatograms that serves as an indicator of extensive biodegradation and suggests that no further degradation was likely (Yang et al., 2011; Crosby et al., 2013). Although resins and asphaltenes in the bitumen are expected to remain recalcitrant over long time periods, any saturates and aromatics would be expected to biodegrade more rapidly. A 30-day laboratory biodegradation experiment was conducted using residual oil in sediment (19-20 months after the spill) from the Enbridge oil spill in the Kalamazoo River (US EPA, 2013a), where approximately 25% of the total petroleum hydrocarbons (TPH) degraded, mostly in the first 14 days. However, the decreasing rate of biodegradation over the 30-day period suggested that the majority of the spilled oil would not degrade over time scales of at least a few months in spite of the experiment's favorable conditions for bacterial activity. This is consistent with the work of King et al. (2014), where first-order rate constants were 0.0011 and 0.0014 d^{-1} for alkanes using Access Western Blend and Cold Lake Blend; and for PAHs, the corresponding rate constants were 0.0011 and 0.0005 d^{-1}. Similarly, Coblani et al. (2015) found that for diluted bitumen, with and without chemical dispersants, alkanes were almost completely degraded for fresh and saltwater, whereas aromatics persisted over a 42-day period. These limited studies highlight that dilbit biodegradation warrants further study to better ascertain the biodegradation rates, environmental factors and compositional variability (Wang and Fingas, 1996).

Compared to conventional crudes, middle distillates and heavy fuel oils, very little is known about the toxicity of dilbits and synthetic crudes derived from bitumen (Dew et al. 2015). Previous studies have reported the toxicity of bitumen, bitumen extracts, or process water from the Alberta tar sands region of Canada (e.g., Colavecchia et al. 2004; Alharbi et al. 2016; Bauer et al. 2017). However, aquatic toxicity data are extremely limited for dilbit, with only four published studies to date (Madison et al. 2015, 2017; Alderman et al. 2016; Philibert et al. 2016). The need for dilbit toxicity data has been highlighted in a number of comprehensive reviews (Dupuis and Ucan-Marin, 2015; NAS, 2016; Lee et al. 2015), including the need for baseline toxicity for a range of dilbits for application in hazard assessment and comparison to other oil products (Dew et al., 2015). Of the published dilbit toxicity studies, none have reported data for standard aquatic species determined with conventional test methods. In the first study of dilbit toxicity in fish, Access Western Blend dilbit caused developmental toxicity and oxidative stress in medaka (*Oryzias latipes*) at total PAH concentrations of 10 to 100 ug/L (Madison et al., 2015). Alderman et al. (2016) reported biomarker responses and impaired swimming performance in salmon early life stages

exposed to 4 to 67 total PAHs from Cold Lake Blend. Philibert et al. (2016) reported similar developmental toxicity, impaired avoidance behavior, and reduced swimming performance in zebrafish (*Danio rerio*) exposed to dilbit and crude oil, where adverse effects were more strongly associated with monoaromatics (15-20 mg/L) rather than PAHs (50-200 ug/L). In the most recent report of dilbit toxicity, physically and chemically dispersed Cold Lake Blend caused developmental toxicity in medaka at concentrations of 3 and 0.1 ug/L total PAHs, respectively (Madison et al. 2017).

Recent reviews produced in collaboration by government agencies, the National Academies of Science, academics and industry highlight the pressing need to better understand the behavior and potential impacts of dilbit spills over land and water. To address knowledge gaps pertaining to the behavior and fate of spilled diluted bitumen, this report summarizes research conducted within the US EPA ORD on the chemical characterization, dispersion effectiveness, biodegradation and toxicity of two types of diluted bitumen- Western Canadian Select and Cold Lake Blend. These studies are being published in Deshpande (2016); Deshpande et al (2017); Barron et al. (2017). The **<u>objectives</u>** of this research were to:

1. Characterize Cold Lake Blend (CLB) and Western Canadian Select (WCS) dilbit products (unweathered) via chemical and physical properties and dispersion effectiveness.
2. Evaluate the biodegradation of alkanes and aromatics of CLB and WCS dilbit as function of two temperatures (5 and 25 °C) using two freshwater cultures: one consortium acclimated to dilbit (Kalamazoo River Enrichment, KRE) and other consortium enriched on soil contaminated with conventional hydrocarbons from the Ohio River (Anderson Ferry Enrichment, AFE); and analysis of microbial community structure via genomic sequencing.
3. Determine the acute and sublethal toxicity of unweathered and weathered CLB and WCS dilbits to standard aquatic test organisms: the freshwater invertebrate *Ceriodaphnia dubia*, the freshwater fish *Pimephales promelas* (fathead minnow), the saltwater invertebrate *Americamysis bahia* (mysid), and the saltwater fish *Menidia beryllina* (inland silverside); using conventional slow-stir Water accommodated fractions (WAF) and static or static renewal methods to allow comparison to the broader literature on the toxicity of oil products.

Results of this work serve to gain insight on the behavior, fate and potential impacts of spilled diluted bitumen in the environment. Such research is needed to better prepare emergency responders for remediation options and the persistence of dilbit in water and sediments. Increasing the knowledge base of dilbit has application in the risk assessments of spills, and allows comparison of the relative hazards of dilbit products to conventional oil products.

2. Methods

Experiments utilized Cold Lake Blend (CLB) and Western Canadian Select (WCS) dilbits obtained from Crude Quality (Edmonton, Alberta, Canada). Subsamples were artificially weathered by nitrogen gas stripping until no change in volume was observed to yield 'weathered' oil for this study. Alaskan North Slope 521 (ANS) and Prudhoe Bay (PBC) crude oils were obtained from the US EPA laboratory stock and tested without weathering.

2.1. Diluted Bitumen Dispersion Effectiveness

2.1.1. Baffled Flask Test Approach

The Baffled Flask Test (BFT) developed by the EPA (Sorial et al., 2004) provides a measure of dispersion effectiveness for a given oil with and without a chemical dispersant. The test utilizes a 150-mL screw-cap trypsinizing flask (an Erlenmeyer flask with baffles), modified by the placement of a glass stopcock near its bottom so that a subsurface water sample can be removed without disturbing the surface oil layer. *Instant Ocean* artificial seawater (Aquarium Systems; Mentor, OH) at salinity of 34 ppt (120 ml) was placed in the baffled flask. 100 µL oil was pipetted directly onto the seawater surface with an Eppendorf repeater pipettor (set at stop 1 using a 5 mL tip). A volume of 4 µL of chemical dispersant was pipetted onto the center of the oil slick (pipettor set at stop 2 using a 100-µL tip), ensuring that the dispersant contacted the oil prior to the water, yielding a dispersant-to-oil (DOR) ratio of 1:25. The flask was mixed on an orbital shaker set at a rotation speed of 250 rpm for 10 min., to receive moderate turbulent mixing (Kaku et al., 2006). At the end of the mixing period, the flask was removed from the shaker and allowed to remain stationary for a quiescent period of 10 minutes. A 30 mL sample volume was collected without disturbing the flask contents in a 50 mL graduated cylinder by opening the stopcock at the bottom of the baffled flask. The sample was transferred to separatory funnel for liquid-liquid extraction; 3 times with 5 mL fresh Dichloromethane (DCM, pesticide quality). The BFT was conducted with 6 replicates and method blanks.

Dispersed oil concentrations were analyzed by UV-visible absorption spectrophotometry (Shimadzu UV-1800) using standard transmission-matched quartz 10-mm path length rectangular cells. Measurements at 340, 370, and 400 nm wavelengths were collected (Fingas et al., 1987). Oil extraction standards in DCM were prepared for each oil with and without dispersant by combining 2 mL oil with 18 mL DCM. A volume of 80 µL dispersant was added to the oil–dispersant stock solutions to give a DOR of 1:25. Stock solution concentrations were determined by mass measurements after each addition. To generate a 6-point calibration curve, a specific volume of the stock standard solution was added to 30 mL synthetic seawater in a 125 mL separatory funnel. These stock solution volumes were adjusted so that the absorbance readings fell within the linear dynamic range (LDR) of the spectrophotometer. Triplicate liquid / liquid extractions of samples were then performed by using 5 mL of DCM for each extraction and adjusting the final extract to 20 or 25 mL (adjusted to maintain the LDR).

2.1.2. Analysis of Extracts

Absorbance at three discrete wavelengths (340, 370, and 400 nm) was used to calculate the area under the absorbance vs. wavelength curve by applying the trapezoidal rule according to the following equation:

$$Area = \frac{(Abs_{340} + Abs_{370}) \times 30}{2} + \frac{(Abs_{370} + Abs_{400}) \times 30}{2} \qquad (1)$$

Area was then used to calculate the Total Oil Dispersed and then the percentage of oil dispersed (%OD), based on the ratio of oil dispersed in the test system to the total oil added to the system, as follows:

$$TotalOilDispersed(g) = \frac{Area}{CalibrationCurveSlope} \times V_{DCM} \times \frac{V_{tw}}{V_{ew}} \qquad (2)$$

where: V_{DCM} = volume of DCM extract, V_{tw} = total volume of seawater in flask, and V_{ew} = total volume of seawater extracted, and

$$\overline{\%OD}_d \ or \ \overline{\%OD}_c = \frac{TotalOilDispersed}{\rho_{oil} \times V_{oil}} \qquad (3)$$

where: $\overline{\%OD}_d \ or \ \overline{\%OD}_c$ = average %oil dispersed by chemical dispersant (d) or physically (c), ρ_{oil} = density of the specific test oil, g/L, and V_{oil} = volume (L) of oil added to test flask (100 µL = 10^{-4} L).

The reported dispersion effectiveness (DE) value is the lower 95% confidence level (LCL95) of the independent replicates, calculated from:

$$LCL95_d \ or \ LCL95_c = \bar{x} - t_{n-1,1-\alpha} \left(\frac{s}{\sqrt{n}} \right) \qquad (4)$$

where: LCL95 is the lower 95% confidence level for chemically dispersed (d) and physically dispersed (c) oil, $\bar{x}$ = mean dispersion effectiveness of the n = 4 replicates, s = standard deviation, $t_{n-1,1-\alpha}$ = 100 x (1 – α)th percentile from the t-distribution with n-1 degrees of freedom, and α = 0.05. Prior to conducting the statistical comparisons, the replicates within a given treatment were subjected to an outlier test, the Grubb's Test or Maximum Normal Residual test (Grubbs, 1969), and if an outlier (p < 0.05) was detected, an additional replicate was analyzed to maintain the required four replicates.

2.1.3. Droplet Size Distribution

Dispersions generated with the BFTs were analyzed by a Laser In Situ Scattering and Transmissometry sensor (LISST 100X; Sequoia Scientific) for droplet size distribution and particle concentration operated in bench top mode. Data processing follows Li et al. (2009), where concentrations are normalized to the maximum value to allow for comparison between dispersions.

2.2. Diluted Bitumen Biodegradation Study

2.2.1. Chemicals and Reagents

Fresh CLB and WCS dilbits were used in the biodegradation experiments. Prudhoe Bay Crude (PBC) and Alaskan North Slope 521 (ANS) were used to represent conventional crude oil. Mineral salts, dichloromethane (DCM), and hexane were acquired from Fisher Scientific (Pittsburg, PA, USA).

2.2.2. Media

Bushnell Hass broth was used as freshwater media. It was prepared by dissolving the required amounts of mineral salts in distilled water. The concentrations of each salt (expressed in g/L) were magnesium sulfate (0.2), calcium chloride (0.02), monopotassium phosphate (1.0), dipotassium phosphate (1.0), ammonium nitrate (1.0), and ferric chloride (0.05). This solution was autoclaved at 120 °C for 15 min in batches of 1 L. The pH for this media was 7.

2.2.3. Microbial Enrichment

Cultures were enriched on hydrocarbon impacted sediments isolated from two different locations. One set of sediments was collected from the Ohio River, downstream of fuel tanks at Anderson Ferry (Cincinnati, Ohio, USA). The other batch of sediments was obtained from the dredging operations following the Kalamazoo River Enbridge Energy Spill. These cultures were named after the locations from where they were acquired: Anderson Ferry (AFE) and Kalamazoo River (KRE) culture. AFE and KRE were enriched on ANS and dilbit, respectively as the carbon source in Bushnell Haas broth for a month. KRE mixed culture was enriched at both 5 (cryo) and 25 ºC (meso), while the AFE was grown at 25 ºC. After 30 days of incubation, cultures were centrifuged, washed with saline and mixed with 10% glycerol before storing at -80 ºC. On the day of experimental set up, enrichment stocks were thawed to room temperature and re-suspended in sterile saline before use.

2.2.4. Microcosm Design

Experiments were set up to examine biodegradation of three types of oils at 5 and 25 ºC using microbial consortia obtained from different locations as summarized in Table 2.1. Sampling days at 25 ºC occurred at days 0, 2, 4, 8, 12, 16, 20, 28, 35, 42, 54, and, 60. The 5 ºC experiments were analyzed on days 0, 2, 4, 8, 16, 24, 32, 40, 48, 56, 62, and, 72. To account for any possible abiotic losses, triplicate killed controls (KCs) containing 500 mg/L of sodium azide were also included and sampled at the end of each experiment. Each flask containing 100 mL of broth was spiked with 0.07 g of oil and was inoculated with 0.5 mL of the culture. These microcosms were then placed on a rotary shaker and mixed at 200 rpm in temperature controlled rooms for the duration of the experiments.

Table 2.1. Summary of Biodegradation Experimental Design.

Test	Temperature	Treatment	Sampling Events	Sample Replicates	Total Experimental unit (EU)
1	5°C	Oil	12	3	36
2	5°C	Kill Control	1	3	3
				Subtotal EU's	39
3	25°C	Oil	12	3	36
4	25°C	Kill Control	1	3	3
				Subtotal EU's	39
		Total EU's for one oil and one enrichment			78

2.2.5. Oil Extraction and Analysis

On every sampling day, three flasks were sacrificed per treatment. Oil was extracted with DCM. Obtained extracts were filtered through anhydrous sodium sulfate to remove any water and then concentrated to reduce the volume under nitrogen. Residual hydrocarbons were measured with an Agilent 7890A Gas Chromatograph with an Agilent 7000 mass selective detector triple quadrupole. The chromatograph was equipped with a general purpose low bleed capillary column (DB-5, 30 m × 0.25 mm and 0.25 µm film thickness, J&W Scientific) and a split/splitless injection port operated in the splitless mode. Multi Reaction Monitoring (MRM) mode was used for analyte detection. The targeted analytes included 28 alkanes ranging in carbon number from n-C10 to n-C35 plus pristane, and phytane, while the aromatics targeted were the 2- ,3-, and 4-ring polycyclic aromatic hydrocarbons (PAHs) [naphthalenes (NAP), phenanthrenes (PHE), fluorenes (FLU), dibenzothiophenes (DBT), naphthbenzothiophenes (NBT), pyrenes (PYR), and chrysenes (CHY)] along with their alkylated homologs. Concentrations of individual analytes were added together to calculate total alkanes and total PAH content. Total Petroleum Hydrocarbons (TPH) were determined with a GC flame ionization detector (GC-FID) according to the EPA Method 8015C.

First order biodegradation rate coefficients were determined by non-linear regression using GraphPad Prism 7. An analysis of variance (ANOVA) was conducted to test the following null hypotheses: (1) no differences exist in the biodegradability of select fractions of dilbit and conventional crude oil, (2) all the enrichments metabolize select fractions of the crude in a similar fashion, and (3) temperature does not affect biodegradation of crude.

2.2.6. DNA Extraction and Sequencing

Samples were collected for molecular analysis on days 0, 2, 16, 35, and 60 for 25 ºC and on days 0, 2, 16, 40, and 72 for 5 ºC. On these sampling days, pellets of biomass were obtained by spinning 1.5 mL of media. Following the manufacturer's instructions, DNA was isolated with the PowerLyzerTM PowerSoil® DNA kit (MoBio Laboratories, Solana Beach, CA). The DNA extracts were then amplified with barcoded primers 515F/806R. Polymerase chain reaction (PCR) conditions used were as follows: denaturing for 3 min at 95 °C, 35 cycles at 95 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, followed by 10 min of final primer extension at 72 °C. The PCR products were visualized on an agarose gel to confirm product sizes and then pooled in an equimolar ration. Next generation sequencing was performed on an Illumina MiSeq sequencer (Illumina, San Diego, CA) by using pair-end 250 bp kits at the Cincinnati Children's Hospital DNA Core facility. Sequence reads were processed with MOTHUR v1.25.1.

2.3. Diluted Bitumen Toxicity to Standard Aquatic Test Species

2.3.1. Test Organisms, Conditions and WAF Preparation

Test organisms (Figure 2.1) were cultured in either moderately hard reconstituted synthetic fresh water (*C. dubia, P. promelas*) or 20 parts per thousand (ppt) synthetic sea water (*A. bahia*) following standard methods (USEPA 2002a; 2002b; 2002c). *M. beryllina* were obtained from a commercial supplier and acclimated to 20 ppt culture water (USEPA 2002a). Neonatal *C. dubia* less than 24-hour old were used in acute and chronic tests. For the chronic tests, they were also isolated within an eight-hour period (USEPA 2002b, method 1002.0). Larval *A. bahia* were used at 3 to 4 days old in acute tests (USEPA 2002a, method 2007.0), and when 7 days old in chronic tests (USEPA 2002c, method 1007.0). Larval *P. promelas* were used in acute tests when 7 to 12 days old (USEPA 2002a, method 2000.0), and *M. beryllina* were used in acute tests as 10 to 14 day old larvae (USEPA 2002a, method 2006.0).

Test methods were species specific and generally followed U.S. EPA effluent test guidelines as modified for tests with petroleum (Table 2.2). Chambers were covered to minimize loss of volatile hydrocarbons, but were not sealed to allow gas exchange. Static (no test solution renewal) acute tests were either 48 hours (*C. dubia, A. bahia*) or 96 hours (*P. promelas, M. beryllina*) that measured mortality and morbidity (no response to gentle prodding). Chronic tests were 7 day exposures with periodic test solution renewals that measured mortality and morbidity, and either reproduction (*C. dubia*) or growth (*A. bahia*).

The CLB and WCS dilbits were artificially weathered by nitrogen gas stripping until no change in volume was observed. WAFs (water accommodated fractions) were prepared in species-specific fresh or 20 ppt salinity culture water in 4 liter sealed and covered glass jars following the standard slow-stir method (e.g., Barron et al. 2003). Oil was added at either 25 or 50 g/L, stirred to achieve an approximately 20% vortex for 18 hours, then settled for 6 hours. The aqueous phase was removed, serially diluted and used in toxicity tests.
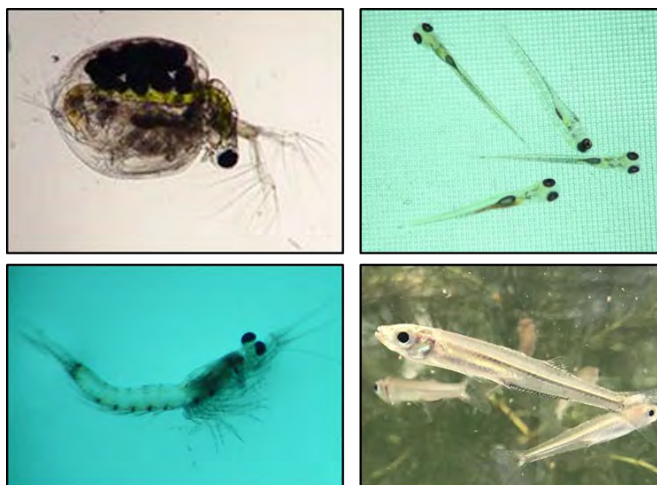
Figure 2.1. Acute and chronic test species: Ceriodaphnia dubia (C. dubia ; top left); Pimephales promelas (fathead minnow; top right); Americamysis bahia (mysid; bottom left); Menidia beryllina (inland silverside; bottom right).

2.3.2. Analytical Chemistry

WAF samples were collected immediately after preparation and analyzed for BTEX, PAHs including alkyl homologs, and TPH as total extracted hydrocarbons. BTEX samples were collected in 40 mL glass head space vials and samples for analysis of PAHs and TPH were collected in 1 L glass jars. Samples were extracted with dichloromethane and analyzed for oil components following SW-846 Method 3500C (USEPA 2007a). Alkane and PAH concentrations were quantified using an Agilent 6890N Gas Chromatograph (GC) with an Agilent 5975 mass selective detector (MSD) and an Agilent 7683 series autosampler, equipped with a DB-5 capillary column by J&W Scientific (30 m, 0.25 mm I.D., and 0.25 μm film thickness) and a splitless injection port following EPA Method 8270D (USEPA 2014). Alkanes consisted of normal aliphatics ranging in carbon number from 10 to 35 as well as branched alkanes (pristine and phytane). Aromatics included 2, 3 and 4 ring PAH compounds and their alkylated homologs (i.e. C_{0-4} – naphthalenes, C_{0-4} -phenanthrenes, C_{0-3} – fluorenes, C_{0-4} dibenzothiophenes, C_{0-3} – napthobenzothiophenes, C_{0-2} -pyrenes, C_{0-4} – chrysenes). TPH in solvent extracts were determined with an Agilent 7890B GC equipped with a flame ionization detector (FID) and 7693 autosampler following EPA Method 8015C (USEPA 2007b). Concentrations of all the individual alkanes and PAHs were summed up to measure total alkane and PAH concentrations. BTEX were measured using an Agilent 7890A GC with a 5975C MSD with Triple Axis Detector and CombiPal autosampler (CTC Analytics) following EPA Method 524.3 modified to perform head space analysis instead of purge and trap (USEPA 2009). All reported hydrocarbon measurements met EPA method quality assurance and quality control requirements, including precision, reproducibility and required detection limits.

2.3.3. Statistical Analyses

Median and 20% lethal concentrations for acute tests (LC50/LC20) were calculated from a log-logistic regression using the drc package in R Statistical Software (Ritz and Streibig 2005) as a percent WAF value. Percent WAF values were then multiplied by initial test stock solution concentrations for BTEX, total PAH (tPAH; sum of detected analytes and homolog groups), and TPH to determine lethal concentrations for each test species, dilbit, and WAF loading level. The average TPH concentration measured in control samples was subtracted from stock solution measurements before determining effective concentrations. For 7 day chronic tests, no observed effect concentrations (NOEC) and 25% inhibition (IC25) values were calculated using the geometric mean concentration of BTEX, PAH, and TPH in test specific stock solution WAF samples. Relationships between species-specific dilbit toxicity and concentrations of TPH, PAH, or BTEX were determined by linear regression analysis using r-squared and p-value.

Table 2.2. Summary of acute and chronic test conditions for four aquatic species: Ceriodaphnia (*Ceriodaphnia dubia*), mysid (*Americamysis bahia*), fathead minnow (*Pimephales promelas*), and inland silverside (*Menidia beryllina*). *Data and Table Source: Barron et al., 2017.*

Test Parameter	Acute *C. dubia*	Acute *A. bahia*	Acute *P. promela*	Acute *M. beryllina*	Chronic *C. dubia*	Chronic *A. bahia*
Test method	EPA-821-R-02-012 Method 2002	EPA-821-R-02-012 Method 2007	EPA-821-R-02-012 Method 2000	EPA-821-R-02-012 Method 2006	EPA-821-R-02-013 Method 1002	EPA-821-R-02-014 Method 1007
Test type	Static	Static	Static	Static	Static renewal	Static renewal
Test duration	48 hours	48 hours	96 hours	96 hours	7 days	7 days
Salinity	NA	20 ± 2‰	NA	20 ± 2‰	NA	20 ± 2‰
Renewal	NA	NA	NA	NA	Daily	Daily
Temperature	25 ± 1 °C. Test temperatures must not deviate (maximum minus minimum temperature) by more than 3 °C during the test.					
Light quality	Ambient laboratory illumination					
Light intensity	10–20 (E/m2/s)					
Photoperiod	16 h light, 8 h darkness, with phase in/out period recommended					
Test chamber size	30 mL	500 mL	1 L	1 L	30 mL	500 mL
Test solution volume	20 mL	200 mL	200 ml	200 mL	20 mL	200 mL
Age of test organism	<24 hours	1–5 days	1-14 days	9–14 days	<24 hours[1]	7 days
Organisms per test chamber	5	10	10	10	1	5
Replicate chambers per concentration	4	3	3	3	10	8
Feeding regime	Refer to specific feeding procedures provided in each test method					
Aeration	None, unless DO falls below 4.0 mg/L, then aerate all chambers. Rate: <100 bubbles/minute					
Phys. / Chem. Measurements	Daily temperatures were measured in one replicate for each test concentration. Exposure test solutions analyzed daily for pH, dissolved oxygen, and conductivity / salinity.					
Test conc.	5 exposure concentrations and a control					
Test acceptability	≥90% control survival	≥90% control survival	≥90% control survival	≥90% control survival	≥80% control survival[2]	≥80% control survival[3]
1. Released within an 8-hour period; 2. Minimum 15 young per surviving female; 3. Minimum weight of 0.2 mg per adult in surviving controls.						

2.4. Quality Assurance Summary

This multidisciplinary research project was a collaborative effort of the EPA Office of Research and Development (ORD) national research laboratories NRMRL and NHEERL. Research was conducted under approved Quality Assurance Project Plans. The EPA Quality system is integral to this effort, providing policy and procedures which are implemented in all aspects of the project to ensure that the data generated from each discipline would be of a type and quality necessary and sufficient to achieve project objectives.

The EPA Quality System encompasses management and technical activities related to the planning, implementation, assessment and improvement of environmental programs that involve:

- the collection, evaluation and use of environmental data

- the design, construction and operation of environmental technology

Consistent with the requirements of the EPA Quality system, the participating EPA organizations have implemented Quality Management Plans to define the specific processes and procedures that each EPA organization uses to ensure implementation of the EPA Quality system.

To that end, the following Quality Assurance (QA) tools were implemented during the project:

- A systematic planning approach was implemented to develop acceptance or performance criteria for all work covered by the EPA Quality System, defined in the Quality Assurance Project Plan (QAPP). QAPPS were developed and approved for use by the EPA Quality staff for each project effort, before any data collection activities were initiated in the field or laboratory. QAPPs that were developed and implemented for this project are identified in the relevant sections of this report and in the references section.

- Standard Operating Procedures (SOP) were implemented for all applicable field and laboratory activities, to ensure consistency in the collection of samples, operation of environmental technologies, and generation of environmental data in the field and in the laboratory.

- Appropriate training was provided for staff to ensure that quality-related responsibilities and requirements as defined in the QAPPs are understood, and that SOPs are implemented for all applicable activities. This ensures that research activities are conducted in a consistent and reproducible manner, with the intent that research data will meet project data quality objectives and/or acceptance criteria for usability to achieve the project objectives.

- Technical assessments were scheduled and performed by EPA and/or contractor quality staff to verify that the QAPP requirements and SOPs were implemented during the project. A technical Systems Assessment was performed by the contractor as required by the QAPP developed for stage 1 of the project.

- Data was reviewed and verified by research staff after collection, and validated by the project leads to ensure that the type, quantity and quality was sufficient to reach conclusions stated in this report and ultimately to achieve project objectives.

Furthermore, it is a requirement that all EPA Quality system elements "flow down" to the contractor support entities. EPA Quality System specifications are incorporated to all applicable EPA-funded agreements, and are defined in 48CFR46. An important element of this for contracted analytical services is certification by an independent accrediting organization such as the National Environmental Laboratory Accreditation Conference (NELAC). This ensures that data is collected according to standard procedures and methodologies under a quality system which is equivalent to ANSI/ASQC E4, which is the basis of the EPA Quality system.

3. Results and Discussion

3.1. Chemical and Physical Characterization of Source Oil

WCS is composed of bitumen from Athabasca basin, whereas CLB is bitumen from the Cold Lake region. Source bitumen from Athabasca is heavier than Cold Lake because of the higher asphaltene content (~17 % versus ~11%, respectively; Figure 1.1) and hence it is blended with conventional crude and synthetic crude oils along with the diluent to meet pipeline specifications (Oil Sands Magazine, 2017). Both WCS and CLB are classified as unconventional heavy crude oil. The physical properties for both CLB and WCS were similar to values previously reported (Alberta Innovates Energy, 2011; NRC, 2013; Canadian Government, 2013). API gravity, density, and sulphur content of two dilbit products were nearly identical, but CLB had higher viscosity and total acid number (Table 3.1). Relative proportion of total alkanes and total PAHs were similar for both dilbit products. The equal saturate and aromatic content of these dilbit products are consistent with the higher densities and viscosities when compared to conventional crude oils. Distribution of *n*-alkanes for the two dilbits was similar, with concentrations decreasing with the carbon chain length. Nevertheless, differences in branched alkane composition were observed namely, *n*-C17/pristane and *n*-C18/phytane ratios for WCS were lower than in CLB (Figure 3.1A). Overall WCS exhibited 22.5% more total alkanes as compared to CLB. WCS presented higher content of naphthalenes with phenanthrenes and dibenzothiophenes being more abundant in CLB. No differences were noted in the concentrations of other PAH groups (Figure 3.1B). Higher quantities of lower molecular weight hydrocarbons like alkanes and naphthalenes in WCS can be one of the reasons for the lower viscosity as compared to CLB (NAS, 2016).

Table 3.1. Physical and chemical properties of CLB and WCS diluted bitumens. Characterization was conducted by Maxxam Analytical International Corp. (Petroleum Technology Center, Edmonton, Canada).

Properties		Cold Lake Blend (CLB)	Western Canadian Select (WCS)
API Gravity at 15 °C	° API	21.7	22.1
Absolute Density at 15 °C	kg/m^3	922.8	920.7
Total Acid Number (TAN)	mg KOH/g	1.03	0.86
Total Sulphur,	mass %	3.68	3.40
Kinematic Viscosity at 15 °C	cSt	230.7	199.7
Total Alkanes	µg/g of dilbit	3100	4000
Total PAHs	µg/g of dilbit	3540	3845
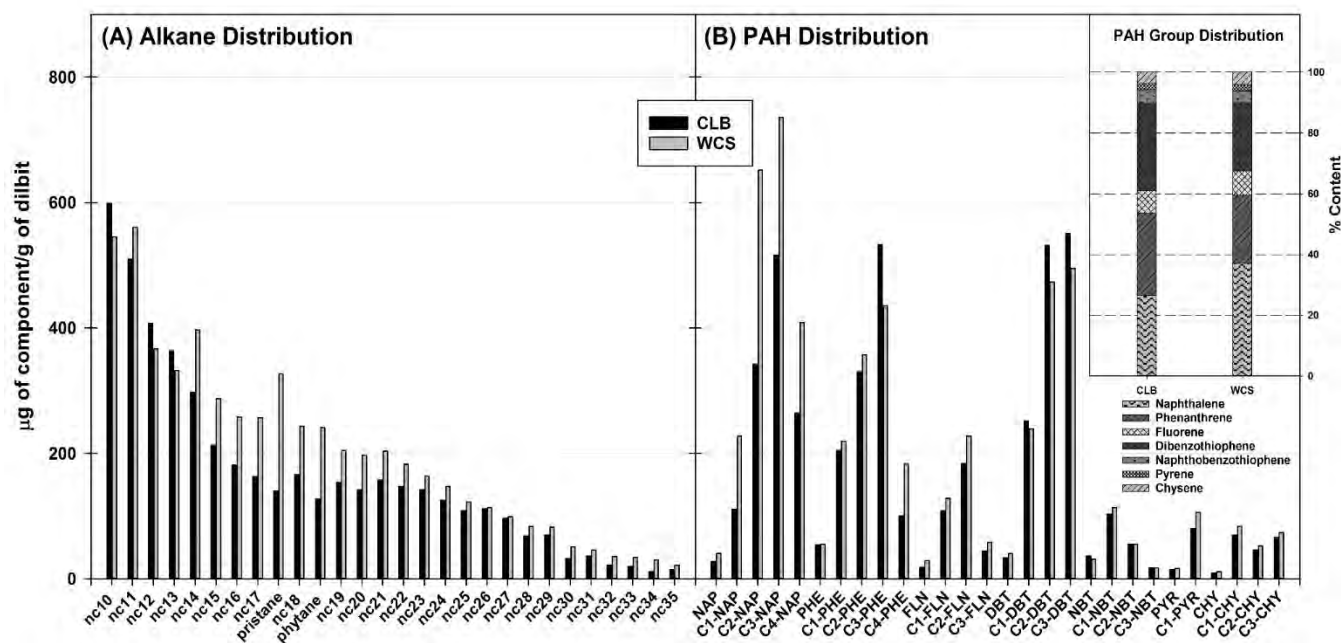
Figure 3.1. Hydrocarbon composition for Cold Lake Blend (CLB) and Western Canadian Select (WCS) dilbits. *Source: Deshpande et al., 2017.*

3.2. Dispersion Effectiveness

Fresh CLB and WCS dilbits were evaluated for dispersant effectiveness using the US EPA Baffled Flask Test (BFT). LCL95 values of percent of oil dispersed using Corexit 9500 and Finasol OSR 52 chemical dispersants are listed in Table 3.2. WCS was better dispersed compared to CLB, regardless of dispersant or temperature. The low dispersion values of CLB also yielded higher variability with standard deviations of approximately 3 for cold temperature and 14.5-17.0 for 25 $^{\circ}$C. The size of the oil dispersed droplets produced during BFTs are presented in Figure 3.2. Volume mean diameter was lower for dispersant treatments and at warmer temperatures. Cold temperature appeared to have a greater influence on CLB dispersion compared to WCS, suggesting that CLB may be less amenable to dispersion at cold temperature.

Table 3.2. Dispersant Effectiveness using the BFT (Baffled Flask Test) expressed by the LCL95 for CLB and WCS using Corexit 9500 and Finasol OSR 52 at 5 and 25 $^{\circ}$C.

| | BFT Dispersant Effectiveness % (LCL95) | | | |
| Temp (°C) | Cold Lake Blend (CLB) | | Western Canadian Select (WCS) | |
	Corexit	Finasol	Corexit	Finasol
5	17.97	17.91	39.18	49.85
25	28.74	30.61	55.79	61.89
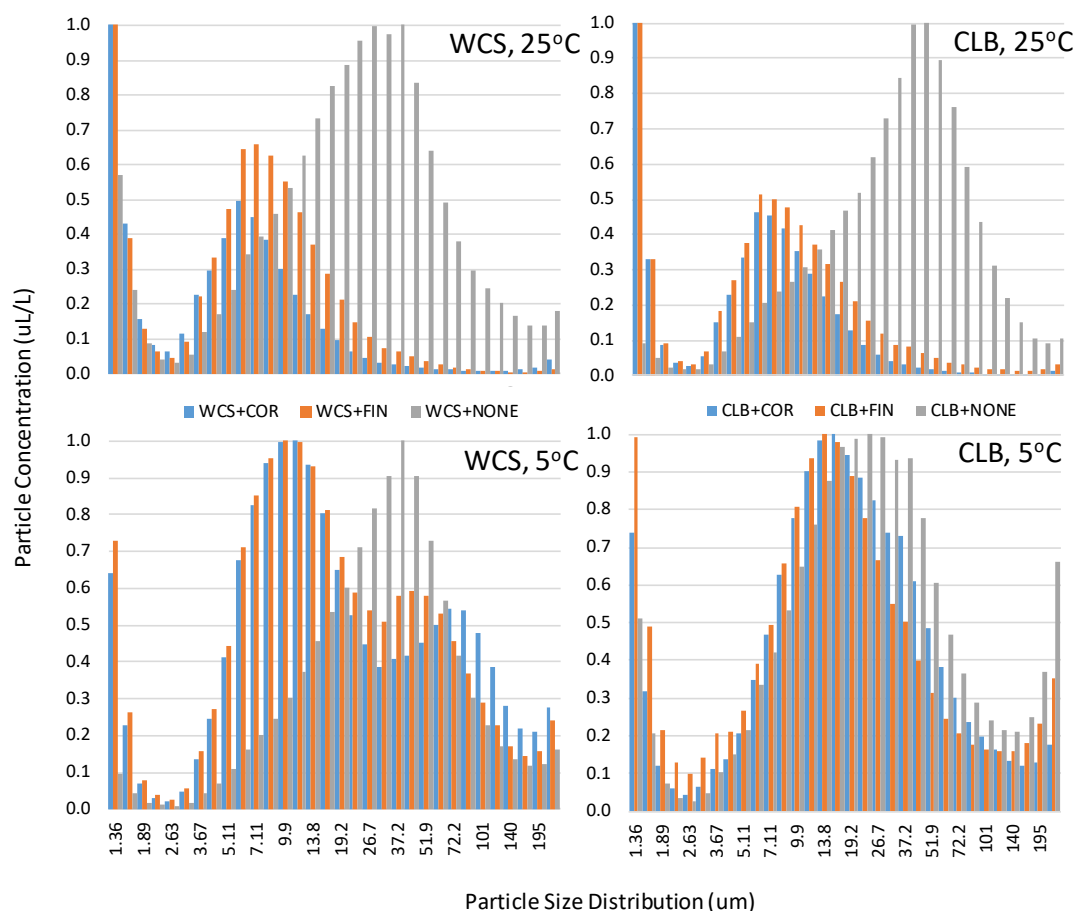
Figure 3.2. Droplet size distribution and particle concentration of Cold Lake Blend (CLB) and Western Canadian Select (WCS) dilbit dispersed in water with and without chemical dispersants (COR: Corexit 9500; FIN: Finasol OSR 52; NONE: no dispersant).

3.3. Dilbit Biodegradation

3.3.1. Alkane and PAH Biodegradation by Kalamazoo River Cultures

Time series removal of total alkanes by Kalamazoo River cultures (KRE) for both dilbits at 5 and 25 °C is shown in Figure 3.3. At lower temperature, the first-order biodegradation rate coefficients were 0.52 ± 0.09 d^{-1} for CLB and 0.48 ± 0.10 d^{-1} for WCS, which were not significantly different (p = 0.9993) (Table 3.3). As expected, rates were higher (2-fold) at 25 °C, with coefficients of 1.13 ± 0.04 and 1.14 ± 0.01 d^{-1} for CLB and WCS, respectively over the course of the experiemnt. No significant difference between dilbit products was observed at 25 °C either (p = 0. 9999). Figure 3.3 illustrates the removal of the sum of all alkanes. As previously reported, linear alkanes are metabolized faster than *iso*-alkanes (Pirnik et al.,

1974; NAS, 1985; Singh et al., 2012). Our study is consistent with these findings, where the meso culture degraded *n*-alkanes within the first two days with the *iso*-alkanes being removed within 8 days. At 5 ºC the *n*-alkanes disappeared by day 8 while *iso*-alkanes persisted for 40 days. The branched alkanes degraded 3.5 times faster at 25 °C than at 5 °C, where degradation rate differences as a function of dilbit type were statistically insignificant at both temperatures (p = 0.5605 (5 °C); p = 0.719 (25 °C)). First-order coefficient rates for individual alkanes were not calculated for the 25 °C treatment as they tend to degrade rapidly. Coefficients calculated for individual alkanes at 5 °C suggested that degradation rates decreased with the increase in carbon number, and no significant difference was observed between dilbit type. Over all, the Kalamazoo River meso culture (25 °C) removed 99.9% of the total alkanes by day 12 (Figure 3.4), while the cryo culture (5 °C) needed more than 40 days for removal of total alkanes owing to the persistence of branched alkanes.

Degradation of both CLB and WCS by the Kalamazoo River culture yielded comparable degradation rates for PAHs, with no significant differences for cryo (p = 0.0944) or meso (p > 0.9999) cultures (Figure 3.3; Table 3.3). At 5 °C, a lag period of 4 days was noted for both dilbits, which was not the case at 25°C. PAHs were metabolized by the meso culture 2 and 2.5 times faster than at 5 °C in the WCS and CLB microcosms, respectively. At experiment completion, approximately 97.5% of total PAHs biodegraded at 25 °C, while 30% remained at 5 °C (Figure 3.4). Examination of specific aromatic groups (2-, 3-, and 4-rings and their alkyl derivatives) as well as individual PAH compounds was conducted. Differences in the rate coefficients of 2- and 3-ring PAHs (i.e., naphthalene (C_{0-1}-NAP), phenanthrene (C_0-PHE), fluorene (C_0-FLU)) between the two dilbits at a given temperature were found to be statistically significant owing to their dissimilar initial concentrations in the source oil. In contrast, the degradation rates of napthbenzothiophenes (NBT), pyrene (PYR), and chrysene (CHY) for the two dilbits were comparable. In the case of naphthalenes, complete removal was achieved at 25 °C by 12 d. At 5 °C, C_{0-3}-NAP disappeared within 40 d and the concentration of C_4-NAP was reduced by 80-85% after 72 d (Figure 3.5). The meso culture almost entirely degraded 3-ring aromatics (PHE, FLU, and DBT (dibenzothiophenes)), while 35-40% of these analytes remained at 5 °C, which mainly comprised C_3 and C_4 types. NBT compounds persisted at 5 °C, whereas at 25 °C, their concentration dropped by 92%. A significant decrease in 4-ring PAH concentration was observed at 25 °C, while at 5 °C < 10% of PYR and CHY degraded. A biodegradation rate comparison matrix (Table 3.4) was created to illustrate significant differences in alkane and PAH biodegradation amongst oil types, cultures and temperatures.

3.3.2. Dilbit Biodegradation by Anderson Ferry Culture

Also investigated in this project was the degradation of WCS using two freshwater cultures: one culture acclimated to dilbit (Kalamazoo River Enrichment, KRE) and other culture enriched on soil contaminated with conventional hydrocarbons from the Ohio River near Cincinnati (Anderson Ferry Enrichment, AFE). Degradation experiments were also conducted using Prudhoe Bay Crude (PBC) with both cultures for comparison. This research is also published in Deshpande, 2016 and Deshpande et al., 2017. PBC is a conventional crude oil exhibiting high aliphatic content with a concentration approximately 6 folds greater than that for WCS and CLB. Thus PBC is not plotted in Figure 3.3 due to concentration scaling issues, but appears in Figure 3.4 as percent removal for comparison to dilbits. The KRE culture nearly

completely eliminated total alkanes at both the temperatures and for both the dilbits and the conventional crude. Biodegradation rates were higher at warmer temperature, and the extent of removal exceeded 99% by day 8 while it took 40 days to achieve the same level of degradation at 5 °C. Similar to KRE, AFE was capable of metabolizing aliphatics completely at 25 °C, but the degradation rates were lower for this enrichment. An acclimation period of 8 days was observed in case of AFE treatment at 5 °C, however almost 98% of total alkanes disappeared by the end of the experiment and residual alkanes mainly comprised of *iso*-alkanes. (Figure 3.3). Biodegradation of branched alkanes showed that rapid and complete removal of pristane and phytane was achieved at 25 °C. After a lag of 8 days, *iso*-alkanes started depleting at 5 °C and trace amounts of branched alkanes were noted at the end of the experiment and the variability in three replicates was higher. At both the temperatures, removal was faster using KME enrichment. Degradation patterns for both the oils were similar for AFE (p = 0.5) as well as KRE treatments (p = 0.42).

The pattern of PAH degradation varied for each treatment (Figures 3.3 and 3.4). Total PAH content of the conventional crude oil was almost twice of the dilbit aromatic content. KRE meso culture metabolized almost 98% of the total PAHs at 25°C, whereas, at 5 °C experiment the extent of biodegradation by KRE culture was observed to be between 75% and 85 %. Conversely, at higher temperature, AFE culture degraded PAHs rapidly till day 20, after which no change in their concentration was observed for WCS or PBC. Approximately 40 % of the initial PAH load remained in WCS while 20 % of residual PAHs were observed in PBC treatments. Lower degradation rates were achieved at 5 °C, with PAH depletion occurring after a 4-day acclimation period. AFE enrichment was able to metabolize 50% PAH content of WCS and 78% in case of PBC.

PAH distribution between WCS and PBC was quite different. Naphthalenes accounted for 58% and 38% of total PAHs in PBC and WCS, respectively. However, quantities of 3 and 4 ring compounds in PBC were less than those for WCS. Biodegradation of individual PAHs and their alkylated homologues suggest that naphthalenes diminished faster and to greater extent as compared to other PAHs, where C_{0-2} -NAP approached below detectable limit for all treatments. Both cryo and meso KRE enrichments were able to metabolize 100% of C_3- NAP and almost 90-95% of C_4- NAP. However, AFE culture did not exhibit extensive degradation of C_{3-4}-NAP for either WCS or PBC. Higher removal of the 3 ring compounds (PHE, FLU, DBT) was noted at 25 °C for the KRE treatments, however, for the AF treatments, concentration decreased until day 20 after which there was no apparent change. Almost 33% and 57% of the residual compounds were seen in PBC and WCS respectively and this fraction mainly comprised of C_{2-4}-PHE, C_{2-3}-FLU, and C_{1-3} DBT. At 5 ºC, AFE metabolized C_{0-1}-PHE, C_0-FLU, and C_0-DBT hydrocarbons, compared to treatments with KRE cryo culture which also showed disappearance of C_{1-2}-FLU, C_0-DBT. No significant change was observed in concentrations of heavier hydrocarbons (NBT, CHY, PYR) in treatments involving AFE or KRE cultures. However, KRE meso culture was able to assimilate 80% of the 4 ring compounds in both WCS and PBC.

Overall, the rate and extent of biodegradation by KRE and AFE was much lower at colder temperatures, similar to previous observations (Atlas and Bartha, 1972; Margesin and Schinner, 2001; Venosa and

Holder, 2007; Campo et al., 2013; Zhuang et al., 2016). This may be due to lower solubilities and crystallization of hydrocarbons, and lower metabolic rates at lower temperatures. Along with the temperature, microbial enrichment had a substantial influence on the degradation of both the crude oils. Significant differences were noted in the nature and extent of hydrocarbon metabolism of the three cultures. KRE meso culture degraded both the crude oils more efficiently. All cultures were able to degrade alkanes, while for PAH degradation, KRE cryo culture was less effective than KRE meso culture. The AFE culture behaved differently at 5 and 25 ºC, taking almost 8 days to adapt to the colder temperature before metabolizing the hydrocarbons. This difference in the microbial activities could be explained by diversity in their community composition, differences in their origin (Kalamazoo River vs. Ohio River), and varying carbon source (dilbit vs ANS) on which they were enriched. Although studies have reported degradation of crude oils using aforementioned dominant microbial communities (Margesin and Schinner, 2001; Cao et al., 2009; Rojo, 2009), AFE was less competent.

3.3.3. Microbial Community Structure during Dilbit Biodegradation

Consortia used in these experiments were isolated from two different locations and were enriched on two different oils at different temperatures prior to the inoculation of the microcosm. Microbial community structure analysis was done to characterize these enrichments and provide composition of the three cultures – KRE meso, KRE cryo and AFE. Analysis of 279,807 16S rRNA gene sequences from all the treatments showed that several bacterial groups were present in the Kalamazoo River meso and cryo enrichments, with similar bacterial composition (Figure 3.6). On the phylum level, no significant difference was observed. *Proteobacteria* was the dominating phyla in all the cultures, additionally, *Actinobacteria* and *Bacteroidetes* were also present in both the KRE cultures. Oil degrading microbial community on the genus level was markedly different between the KRE cultures and AFE. *Acinetobacter* (72%) was the dominant genera in AFE microbial community, whereas high abundance of *Pseudomonas* (13%, 17%), *Rhodococcus* (22%, 26.5%), and *Hydrogenophaga* (15%, 12%) was observed in KRE cryo and meso cultures. Though several other genera were present in these consortia, their abundance was very low as compared to the above mentioned genera.

For experiments using KRE cultures, at the start of experiments (day 0), 10 phyla were detected; dominated by members of the Proteobacteria (74 ± 7 and 73 ± 3% for meso and cryo, respectively), the Actinobaceria (24 ± 7 and 16 ± 4%), and the Bacteroidetes (3 ± 1 and 10 ± 1%). Most of the Proteobacteria in the cryo enrichment was associated with beta-Proteobacteria (41 ± 3%), while in the meso enrichment, gamma-Proteobacteria (41 ± 6%) was the most abundant class. A total of 190 different genera were identified from the DNA extracts obtained at day 0. *Pseudomonas*, *Rhodococcus*, and *Hydrogenophaga* were the prominent genera present in both enrichments, but their abundance varied. For example, 21 ± 4 % of the sequences in the meso enrichment were linked to *Pseudomonas* versus 9 ± 1% in the cryo enrichment. *Rhodocoocus* represented 15 ± 4 and 19 ± 8 % of the sequences in cryo and meso enrichments, respectively. Genera with abundance less than 1% were grouped together and labeled as other. This group represented more than 150 different genera (e.g., *Acinetobacter*, *Pedobacter*, *Achromobacter*, *Aquabacterium*). The microbial community structure of KRE cryo and meso

enrichments changed considerably during experiments, however, there were no observable differences when the two different oil types were compared. For example, by day 2, *Pseudomonas* sp. comprised up to ~ 90% of the total community in meso enrichment, which represented more than a four-fold increase from day 0. The same group decreased in abundance by day 16 (i.e., approximately 60%) followed by a gradual increase. The community profile did not alter significantly from day 16 until the end of the experiment (day 60) in either one of the meso treatments. Even though *Pseudomonas* dominated microbial community in meso consortium throughout the time course, other genera like *Hydrogenophaga*, *Parvibaculum* and unclassified members of *Xanthomonadaceae* and gamma-Proteobacteria showed higher abundance as well. Unlike meso enrichment, no one single genus dominated the cryo enrichment.

While there were no differences between the overall community profiles between two dilbits, differences were noted when temperatures were taken into account. Specifically, no significant differences were detected in observed species when treatments with two different dilbits were compared ($p = > 0.901$). Nevertheless, statistical analysis showed significant differences in Shannon diversity based on temperature ($p < 0.001$), although the difference in observed species was not significant ($p = 0.443$). These results indicate that while similar number of taxa present at 5 and 25 °C, their abundance and evenness varied. Multivariate analysis showed that for a given treatment similar taxa were present and microbial community shifted with time. The changes in community structure were more significant at 5 °C compared to 25 °C. PERMANOVA analysis suggested that there was no significant difference in taxa composition between CLB and WCS ($p = 0.953$) whereas the when two temperatures were compared, the taxa composition was statistically different ($p = 0.001$).

Table 3.3 Percent removal and first order rate constants for total alkanes, total PAHs and TPH degradation. KRE = Kalamazoo River Enrichment; AFE = Anderson Ferry Enrichment; CLB = Cold Lake Blend; WCS = Western Canadian Select; PBC = Prudhoe Bay Crude. *Data Sources: Deshpande, 2016; Deshpande et al., 2017.*

T (°C)	Oil	Enrichment	Total Alkanes				Total PAH				TPH			
			% Removal	k (d⁻¹)	SD	R^2	% Removal	k (d⁻¹)	SD	R^2	% Removal	k (d⁻¹)	Std. Error	R^2
5	CLB	KRE	99.62	0.52	0.09	1.00	65.56	0.05	0.02	1.00	19.75	0.04	0.03	0.83
5	WCS	KRE	99.92	0.48	0.10	1.00	74.19	0.07	0.03	0.99	26.18	0.06	0.01	0.97
5	PBC	KRE	99.68	0.56	0.08	1.00	85.31	0.15	0.09	0.99	56.56	0.06	0.01	0.97
5	WCS	AFE	98.01	0.44	0.23	1.00	55.74	0.04	0.03	0.98	25.83	0.01	0.01	0.94
5	PBC	AFE	99.23	0.24	0.09	1.00	79.18	0.03	0.03	0.98	44.47	0.07	0.02	0.96
25	CLB	KRE	99.97	1.13	0.04	1.00	97.56	0.14	0.03	0.98	69.18	0.07	0.02	0.97
25	WCS	KRE	99.95	1.14	0.06	1.00	97.26	0.16	0.01	1.00	64.32	0.11	0.04	0.96
25	PBC	KRE	99.89	1.53	0.10	1.00	98.47	0.24	0.04	1.00	82.51	0.11	0.06	0.97
25	WCS	AFE	99.18	0.90	0.04	1.00	60.88	0.18	0.06	0.98	25.17	0.12	0.02	0.96
25	PBC	AFE	99.85	1.26	0.09	1.00	84.46	0.31	0.05	1.00	52.21	0.16	0.03	0.96
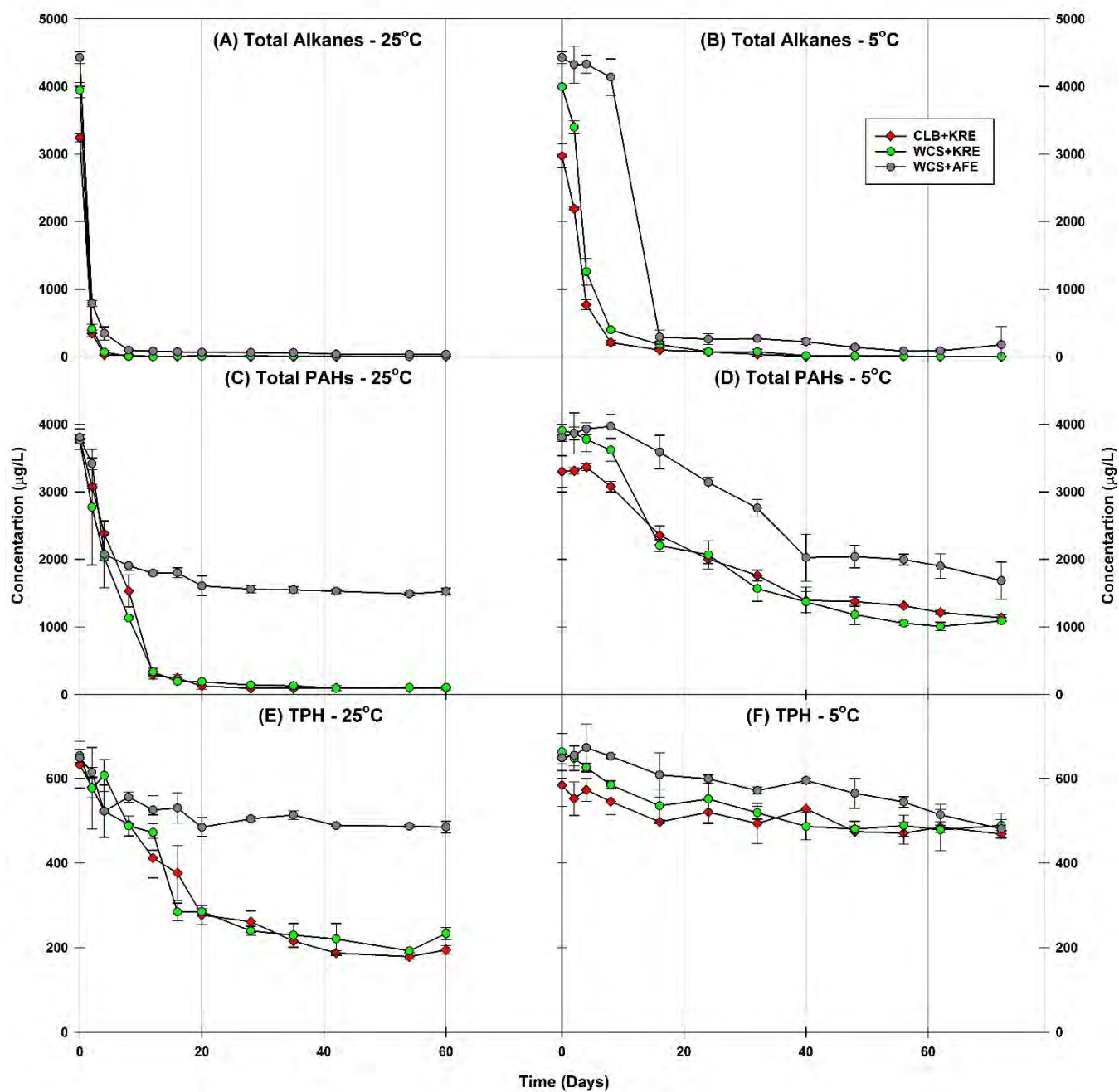
Figure 3.3. Alkanes, PAH and TPH concentration at 25 and 5 °C. KRE = Kalamazoo River Enrichment; AFE = Anderson Ferry Enrichment; CLB = Cold Lake Blend; WCS = Western Canadian Select. *Data Sources: Deshpande, 2016; Deshpande et al., 2017.*
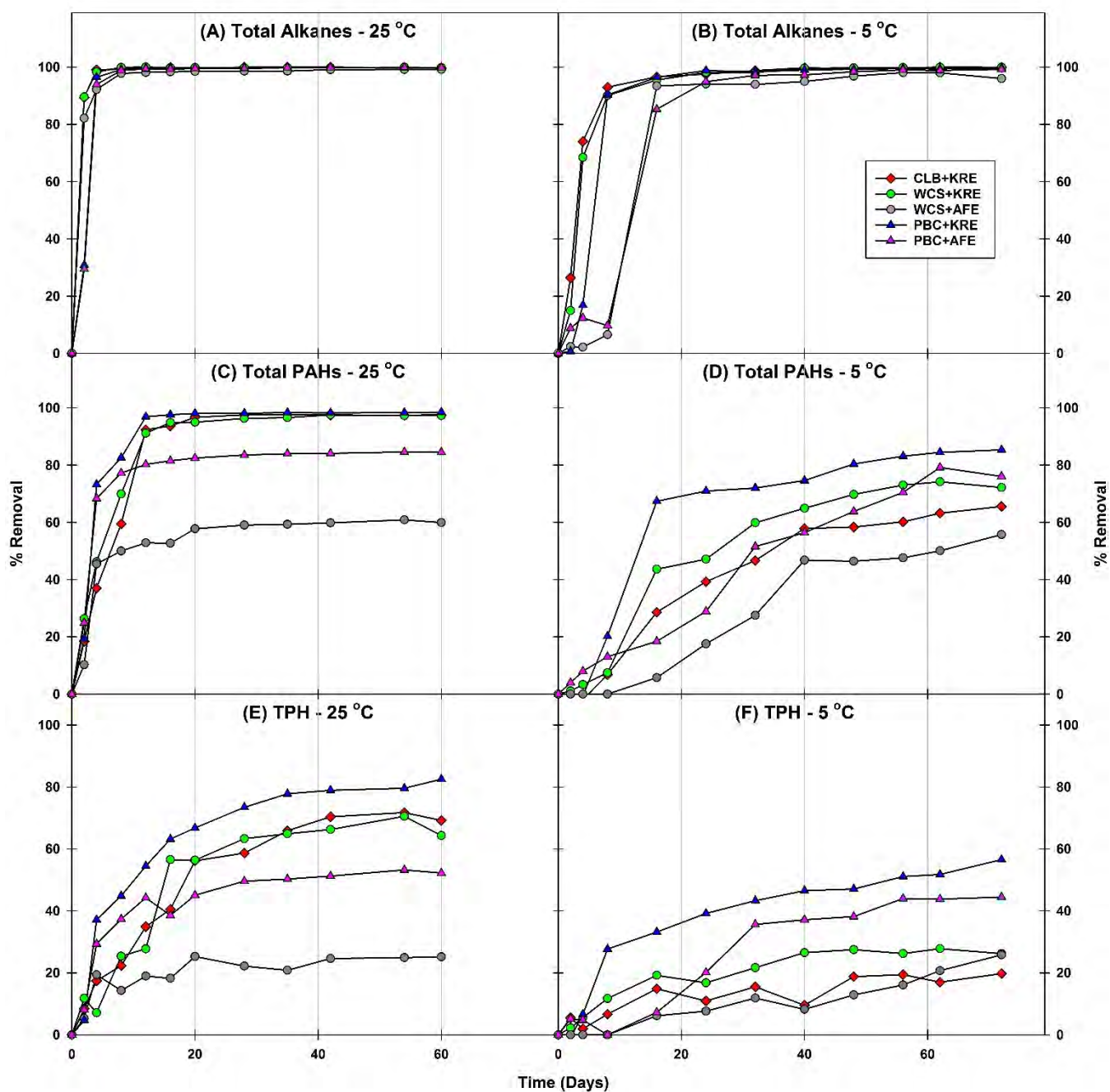
Figure 3.4. Alkanes, PAH and TPH percent removal at 25 and 5 °C. Figure 3.2. Alkanes, PAH and TPH concentration at 25 and 5 °C. KRE = Kalamazoo River Enrichment; AFE = Anderson Ferry Enrichment; CLB = Cold Lake Blend; WCS = Western Canadian Select. *Data Sources: Deshpande, 2016; Deshpande et al., 2017.Data Sources: Deshpande, 2016; Deshpande et al., 2017.*

Table 3.4. Oil biodegradation rate comparison matrix. KRE = Kalamazoo River Enrichment; AFE = Anderson Ferry Enrichment; CLB = Cold Lake Blend; WCS = Western Canadian Select. Red box represents significant difference between treatment and green box represents no significant difference based on ANOVA *p* values. *Data Sources: Deshpande, 2016; Deshpande et al., 2017.*

Degradation Rate Comparison — A – Total Alkanes, P – Total PAH. Red = Significant Difference; Green = No Significant Difference.

Temp	Oil	Enrichment	5 / CLB / KRE		5 / WCS / KRE		5 / PBC / KRE		5 / WCS / AFE		5 / PBC / AFE		25 / CLB / KRE		25 / WCS / KRE		25 / PBC / KRE		25 / WCS / AFE		25 / PBC / AFE	
5	CLB	KRE																				
5	WCS	KRE	A (g)	P (g)																		
5	PBC	KRE	A (g)	P (r)	A (g)	P (r)																
5	WCS	AFE	A (g)	P (g)	A (g)	P (g)	A (g)	P (r)														
5	PBC	AFE	A (r)	P (g)	A (r)	P (g)	A (r)	P (g)	A (r)	P (g)												
25	CLB	KRE	A (r)	P (r)	A (r)	P (r)	A (r)	P (g)	A (r)	P (r)	A (r)	P (r)										
25	WCS	KRE	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (g)	P (g)								
25	PBC	KRE	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (g)	P (r)						
25	WCS	AFE	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (g)	P (g)				
25	PBC	AFE	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)	A (r)	P (r)		

Figure 3.5. Percent removal of individual PAH compounds. *Source: Deshpande et al., 2017.*

Figure 3.6. Microbial Community Structure Analysis during oil biodegradation experiments. KRE = Kalamazoo River Enrichment; AFE = Anderson Ferry Enrichment; CLB = Cold Lake Blend; WCS = Western Canadian Select; PBC = Prudhoe Bay Crude. *Data Sources: Deshpande, 2016; Deshpande et al., 2017.*
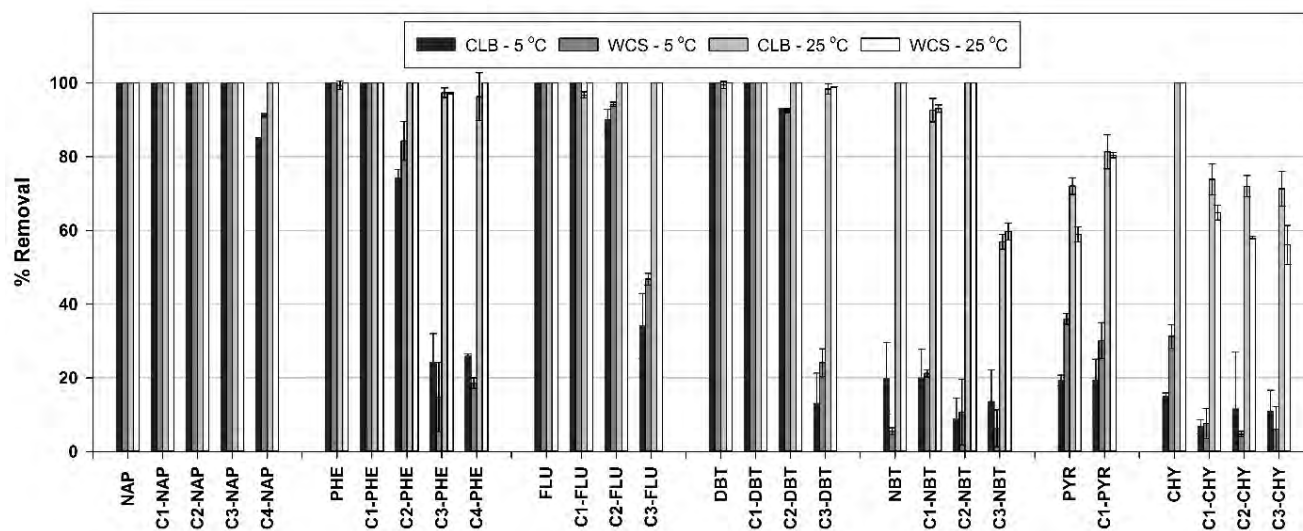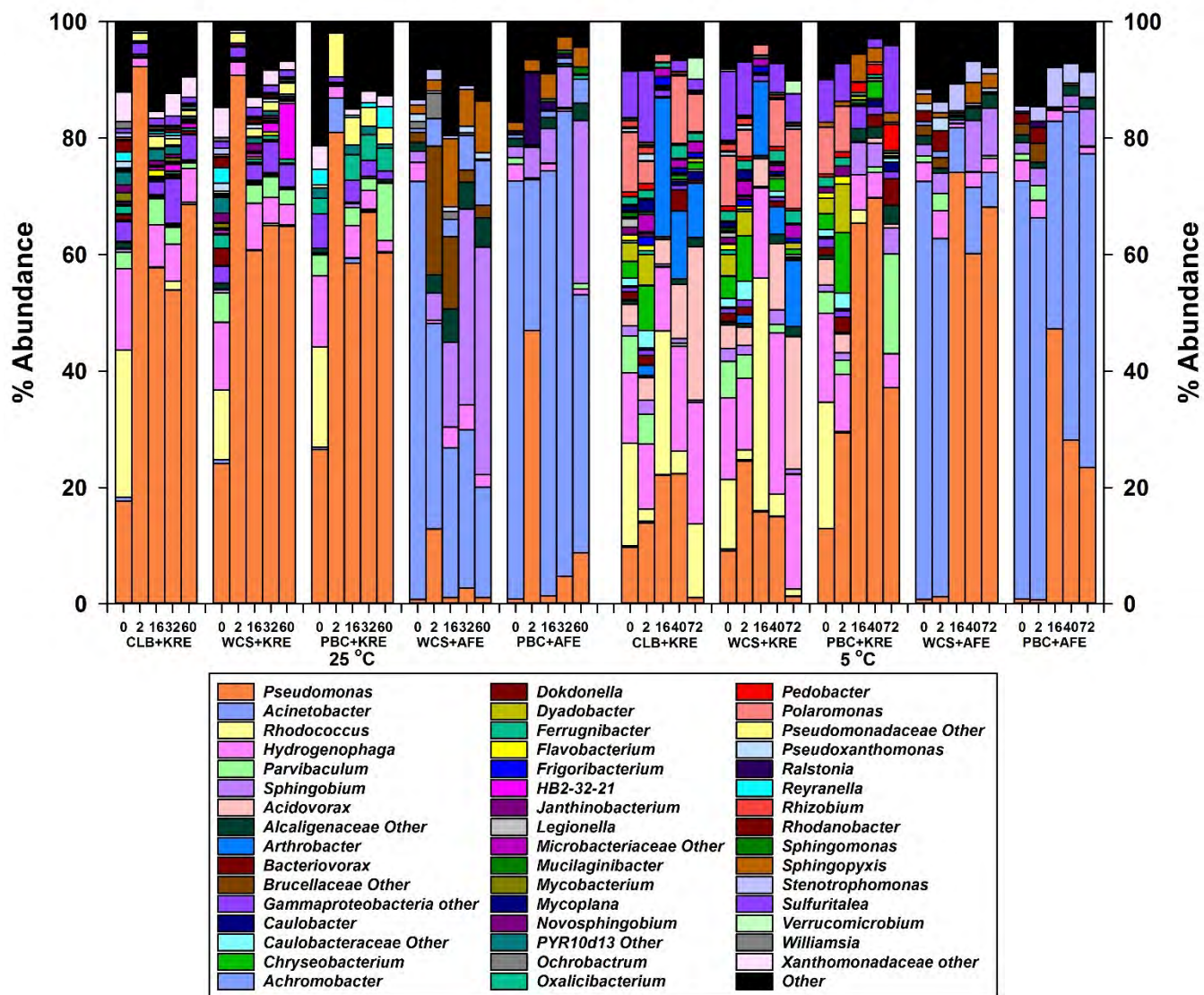
3.4. Toxicity of Dilbit to Standard Aquatic Species
3.4.1. Oil and WAF Chemistry

Chemical analyses of the 100% WAF indicate that total PAH and alkane concentrations in unweathered (fresh) and weathered CLB and WCS were generally similar (Table 3.5). Fresh CLB exhibited higher TPH values (8.36-16.2 mg/L oil), whereas WCS was higher in PAH (18.0-40.0 µg/L oil). CLB and WCS demonstrated similar concentrations of individual PAH analytes and alkyl homologs, where WCS exhibited a larger proportion of N0-4 Naphthalenes (Figure 3.7). Results indicate that Total PAH (17.7 mg/g) and alkane (50.5 mg/g) concentrations in the ANS reference oil were 2 to 10 fold higher than in the dilbits. Mean TPH concentrations in 100% WAF were about 1.5 to 3 fold higher in CLB and WCS (5.9 to 16.2 mg/L) compared to the weathered dilbits (3.9 to 5.6 mg/L) (Table 3.5). Mean PAH concentrations in 100% WAF were similar in fresh or weathered CLB and WCS, and ranged from 13.8 to 40 µg/L. Mean BTEX concentrations in 100% WAF were about tenfold higher in CLB and WCS (4.6 to 11.4 mg/L) than in the weathered dilbits (0.53 to 0.94 mg/L).

Table 3.5. Hydrocarbon concentrations[1] and percent survival of four species in 100% water accommodated fractions (WAF) of two unweathered and weathered dilbits[2] at 24 hours and test end (48 hr invertebrates; 96 hr fish). *Source: Table generated from Barron et al., 2017 data.*

Test WAF[1]	Oil Loading (g/L)	% Survival in 100% WAF		% Survival in 100% WAF		100% WAF concentration[2]		
		24 hour	Test End	24 hour	Test End	BTEX (mg/L)	PAH (ug/L)	TPH (mg/L)
FW WAF		*C. dubia*	*C. dubia*	*P. promelas*	*P. promelas*			
CLB	50	80	65	73	73	7.24	27	16.2
	25	100	85	83	80	11.4	20.5	11.1
CLB W	50	100	100	93.3	93.3	0.76	26.8	5.57
WCS	50	100	95	80	80	5.86	40	10.7
	25	100	85	100	100	5.56	29.4	8.33
WCS W	50	100	85	100	100	0.94	34.9	4.69
SW WAF		*A. Bahia*	*A. Bahia*	*M. beryllina*	*M. beryllina*			
CLB	50	0	0	6	3	5.66	20.7	9.88
	25	10	3	47	47	5.09	13.8	8.36
CLB W	50	86.6	70	100	83.3	0.53	22.1	3.92
WCS	50	0	0	13.3	13.3	4.91	32.6	7.96
	25	70	53	97	97	4.59	18	5.9
WCS W	50	100	100	100	93.3	0.7	28.7	3.93

1. CLB: Cold Lake Blend dilbit; CLB W: weathered CLB; WCS: Western Canadian Select dilbit; WCS W: weathered WCS; FW: freshwater; SW: saltwater.

2. BTEX: benzene, toluene, ethylbenzene, xylenes; PAH: polycyclic aromatic hydrocarbons; TPH: total petroleum hydrocarbons.
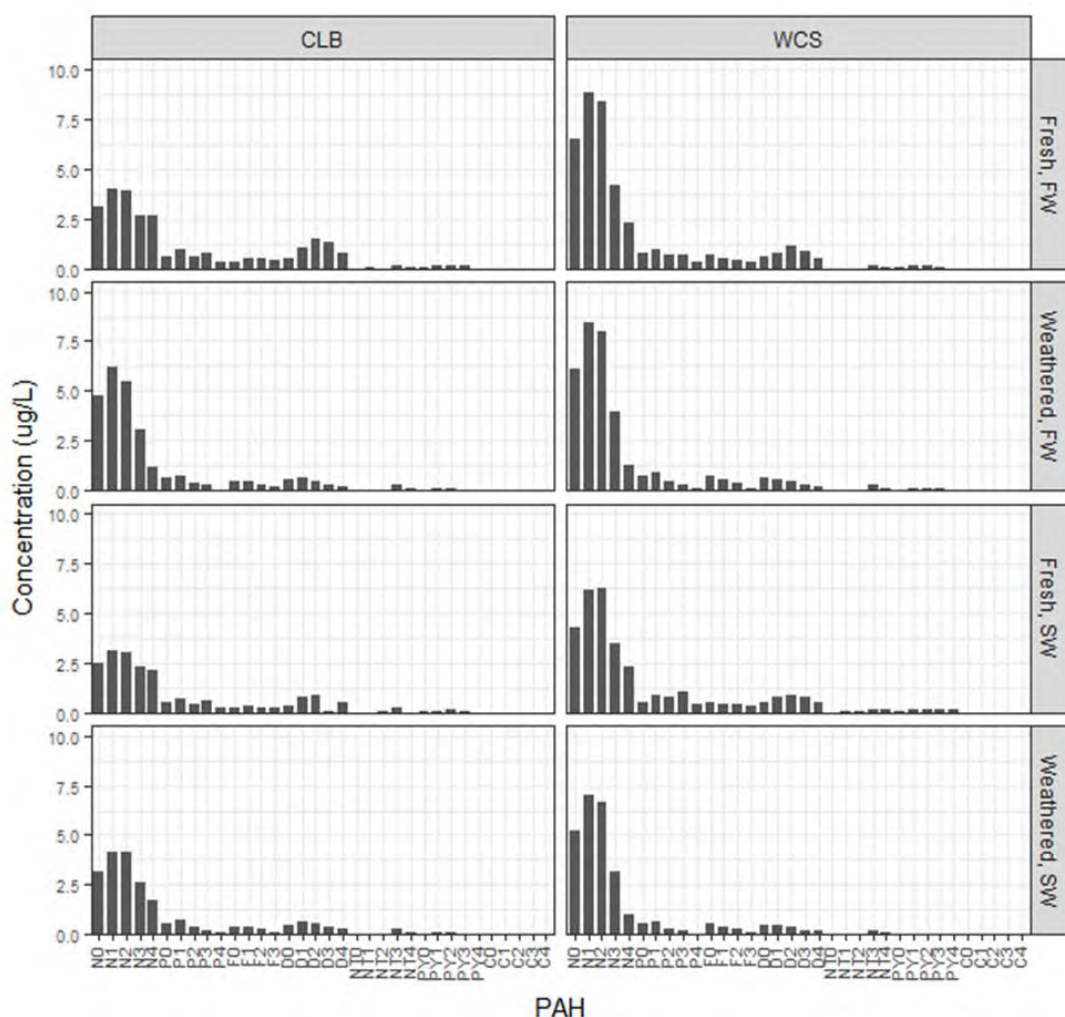
Figure 3.7. Concentration of individual PAH analytes and alkyl homologs in water accommodated fractions (WAF) of fresh (unweathered) and weathered Cold Lake Blend (CLB) and Western Canadian Select (WCS) dilbits. FW = freshwater; SW = saltwater. *Source: Barron et al., 2017.*

3.4.2. Acute and Chronic Dilbit Toxicity

In general, the toxicity of WAF prepared from unweathered and weathered CLB and WCS was similar in all four tested species. LC20 and LC50 values determined at loading rates of 25 or 50 g oil/L ranged from 3.6 to >16 mg/L TPH and 7.4 to >40 ug/L PAH (Table 3.6). Acute toxicity values based on measured BTEX in WAF were lower in weathered CLB and WCS and were substantially more variable (>0.7 to >15.9 mg/L) most likely due to depleted BTEX levels (Table 3.6). TPH concentrations were highly correlated and a significant predictor of acute dilbit toxicity to three of the four test species (excluding *C. dubia*), compared to BTEX and PAH, which were generally not significantly correlated with dilbit toxicity except for mysids (Figure 3.8). Weathered CLB and WCS impaired growth (*A. bahia*) and reproduction (*C. dubia*) at concentrations of 0.41 to 3.5 mg/L TPH, 5.7 to 16 ug/L PAH, and 0.0023 to 1.1 mg/L BTEX (Table 3.7). Acute toxicity values of ANS in the four test species ranged from 2.1 to >5 mg/L TPH, 75 to >107 ug/L

PAH, and >4.3 to >5.6 mg/L BTEX (Table 3.9). All acute and chronic tests met quality control and quality assurance requirements, including control survival and water quality conditions (Table 2.2).

The toxicity of CLB and WCS determined in standard aquatic toxicity tests were generally similar to other oil products based on both TPH and PAH-based measures of effect, including ANS, tested here. For example, TPH-based LC50s for unweathered CLB and WCS determined in mysids (5.6-7.0 mg/L) and menidia (5.9-8.3 mg/L) were consistent with average acute toxicity values for mysids (2.7 mg/L) and menidia (7.5 mg/L) using conventional WAF preparation and toxicity test methods across a range of crude oils (Barron et al. 2013). PAH concentrations impairing survival, growth or reproduction in short term chronic studies with these four North American species (6 to >16 ug/L) were consistent with PAH effect concentrations (3 to 200 ug/L) determined in developmental toxicity studies of dilbits with zebrafish and medaka (Madison et al. 2015, 2017; Alderman et al. 2016; Philibert et al. 2016). PAH-based LC50 values of CLB and WCS (9.8 to >40 ug/L) were also within the range of acute toxicity values of crude oils for a diversity of aquatic species (30-150 ug PAH/L) (Bejarano et al., 2017). The weathered dilbits were chronically toxic, but showed only limited acute toxicity, presumably due to depletion of BTEX. Monoaromatics and napthalenes have long been considered the drivers of acute toxicity of petroleum, including in a recent study of two crude oils and a dilbit (Philibert et al. 2016). Toxicity values were reported for separate analyte groups (BTEX, PAHs), with the recognition that petroleum exposures are mixtures of a group of diverse hydrocarbons that can contribute to toxicity. Consistent with the general literature, effect levels of the dilbits were based on measured concentrations in 100% WAF and did not reflect loss of hydrocarbons over time, thus actual effect levels would be lower than reported here (Redman and Parkerton 2015).

Table 3.6. Acute toxicity of fresh and weathered Cold Lake Blend and Western Canadian Select dilbit to four species as three measures of hydrocarbon exposure.[1] *Data source: Barron et al., 2017.*

Test Species	Weathered State	Test Endpoint	Oil Loading (g/L)	Cold Lake Blend				Western Canadian Select			
				% WAF	BTEX[1] (mg/L)	tPAH[1] (ug/L)	TPH[1] (mg/L)	% WAF	BTEX[1] (mg/L)	tPAH[1] (ug/L)	TPH[1] (mg/L)
Cladoceran (*Ceriodaphnia dubia*)	Fresh	48 hr LC20	50	94.2	6.82	25.4	15.2	>100	>5.86	>40.0	>10.7
			25	68.2	7.74	14.0	7.54	>100	>5.56	>29.4	>8.33
		48 hr LC50	50	>100	>7.24	>27.0	>16.2	>100	>5.86	>40.0	>10.74
			25	70.7	8.02	14.5	7.82	>100	>5.56	>29.4	>8.33
	Weathered	48 hr LC20	50	>100	>0.76	>26.8	>5.57	>100	>0.94	>34.9	>4.69
		48 hr LC50	50	>100	>0.76	>26.8	>5.57	>100	>0.94	>34.9	>4.69
Mysid (*Americamysis bahia*)	Fresh	48 hr LC20	50	67.8	3.84	14.1	6.7	67.8	3.33	22.1	5.37
			25	55.2	2.81	7.63	4.61	91.1	4.18	16.4	5.37
		48 hr LC50	50	70.5	3.99	14.6	6.97	70.5	3.46	23.0	5.61
			25	70.9	3.61	9.80	5.93	>100	>4.59	>18.0	>5.90
	Weathered	48 hr LC20	50	91.1	0.48	20.1	3.57	>100	>0.70	>28.7	>3.93
		48 hr LC50	50	>100	>0.53	>22.1	>3.92	>100	>0.70	>28.7	>3.93
Fathead minnow (*Pimephales promelas*)	Fresh	96 hr LC20	50	96.9	7.02	26.2	15.7	91.1	5.34	36.5	9.78
			25	97.2	11.03	19.9	10.8	>100	>5.56	>29.4	>8.33
		96 hr LC50	50	>100	>7.24	>27.0	>16.3	>100	>5.86	>40.0	>10.7
			25	>100	>11.4	>20.5	>11.1	>100	>5.56	>29.4	>8.33
	Weathered	96 hr LC20	50	>100	>0.76	>26.8	>5.57	>100	>0.94	>34.9	>4.69
		96 hr LC50	50	>100	>0.76	>26.8	>5.57	>100	>0.94	>34.9	>4.69
Inland silverside (*Menidia beryllina*)	Fresh	96 hr LC20	50	61.3	3.47	12.7	6.06	50.7	2.49	16.5	4.04
			25	89.9	4.58	12.4	7.52	>100	>4.59	>18.0	>5.90
		96 hr LC50	50	70.7	4.00	14.5	6.99	73.8	3.62	24.1	5.87
			25	99.1	5.04	13.7	8.28	>100	>4.59	>18.0	>5.90
	Weathered	96 hr LC20	50	>100	>0.53	>22.1	>3.92	>100	>0.7	>28.7	>3.93
		96 hr LC50	50	>100	>0.53	>22.1	>3.92	>100	>0.7	>28.7	>3.93

1. BTEX: benzene, toluene, ethylbenzene, xylenes; tPAH: sum of detected polycyclic aromatic hydrocarbons and homolog groups; TPH: total petroleum hydrocarbons.
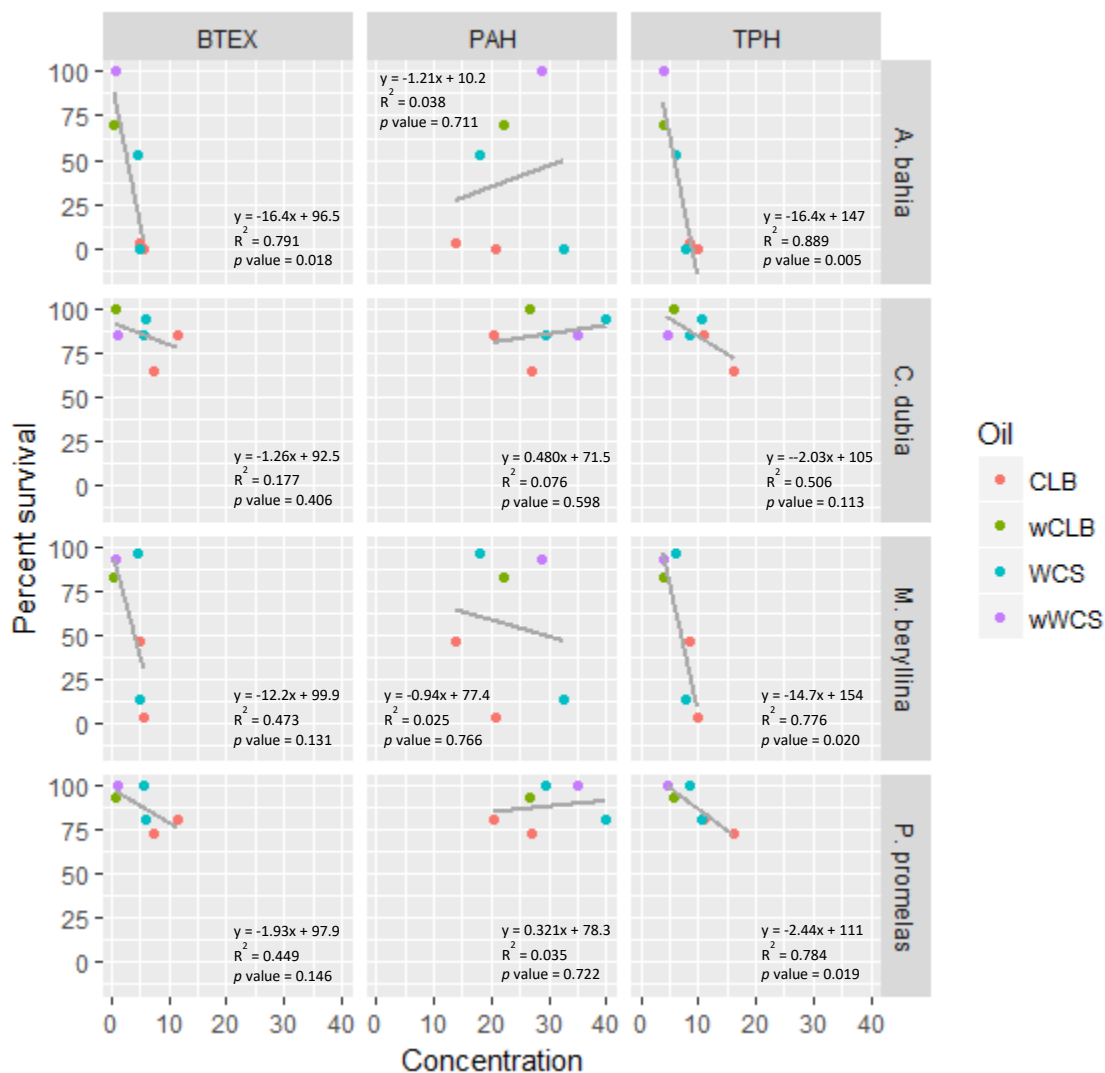
Figure 3.8. Percent survival of four aquatic species exposed to water accommodated fractions of fresh and weathered dilbits: Cold Lake Blend (CLB), weathered CLB (wCLB), Western Canadian Select (WCS), and weathered WCS (wWCS). Test concentrations: BTEX (mg/L); PAH (ug/L); TPH (mg/L). Test species: Ceriodaphnia (*Ceriodaphnia dubia*); mysid (*Americamysis bahia*); fathead minnow (*Pimephales promelas*); inland silverside (*Menidia beryllina*).

Table 3.7. Short-term chronic toxicity of two weathered dilbits to C. dubia and A. bahia as three measures of hydrocarbon exposure.[1] *Source: Barron et al., 2017.*

Test Species	Test Type	Dilbit	Endpoint[2]	% WAF	BTEX[1] (mg/L)	tPAH[1] (ug/L)	TPH[1] (mg/L)
Cladoceran (*Ceriodaphnia dubia)*	7-day survival and reproduction	Cold Lake Blend	NOEC	25	0.088	5.99	1.67
			IC25	52	0.185	12.5	3.49
		Western Canadian Select	NOEC	6.25	0.0003	1.90	0.094
			IC25	53	0.0024	16.0	0.789
Mysid (*Americamysis bahia*)	7-day survival and growth	Cold Lake Blend	NOEC	25	0.0015	3.67	0.262
			IC25	39	0.0023	5.72	0.409
		Western Canadian Select	NOEC	25	0.865	6.15	0.561
			IC25	32	1.10	7.82	0.713

1. BTEX: benzene, toluene, ethylbenzene, xylenes; tPAH: sum of detected polycyclic aromatic hydrocarbons and homolog groups; TPH: total petroleum hydrocarbons.

2. NOEC: no observed effect concentration; IC25: concentrations causing 25% inhibition.

Table 3.8. Acute toxicity of Alaskan North Slope crude oil to four species as three measures of hydrocarbon exposure.[1] *Data source: Barron et al., 2017.*

Test Species	Test Endpoint	% WAF	BTEX[1] (mg/L)	tPAH[1] (ug/L)	TPH[1] (mg/L)
Cladoceran (Ceriodaphnia dubia)	LC20	96.1	5.4	103	4.83
	LC50	>100	>5.6	>107	>5.03
Mysid (Americamysis bahia)	LC20	97.8	5.4	75.3	2.14
	LC50	>100	>4.3	>77.0	>2.19
Fathead minnow (Pimephales promelas)	LC20	>100	>5.6	>107	>5.03
	LC50	>100	>5.6	>107	>5.3
Inland silverside (Menidia beryllina)	LC20	>100	>4.3	>77.0	>2.19
	LC50	>100	>4.3	>77.0	>2.19
1. BTEX: benzene, toluene, ethylbenzene, xylenes; PAH: polycyclic aromatic hydrocarbons; TPH: total petroleum hydrocarbons.					

4. Report Summary

Dilbit presents an increasing environmental concern because of extensive transport in North America, recent spills into aquatic habitats, and limited understanding of environmental fate and toxicity (Dupuis and Ucan-Marin 2015, NAS 2016; Lee et al. 2015). Dilbit is a blend of highly weathered bitumen and lighter diluent oils that contain higher concentrations of asphaltenes (>10%) and lower levels of saturates (~40%) (NAS 2016), with unique properties, including high adhesion, and the potential for rapid weathering, sinking and associating with sediments. Information on dilbit biodegradation, toxicity, dispersion and fate is limited and warrants further study, particularly given the variety of types of dilbit and variability in weathering states in the environment. This research will allow for more informed risk assessments and improved emergency response during dilbit spills in aquatic and terrestrial environments.

This research showed that select fractions of dilbit can be efficiently biodegraded, but under similar conditions, conventional crude oil was degraded more effectively due to the higher content of lighter hydrocarbons. The rates of alkane and PAH biodegradation were comparable for dilbit products, but the extent of degradation was greater for PBC because of the higher concentrations of lighter alkanes. The potential of microbial enrichment to degrade crude oil was highly influenced by temperature as well as the composition. Lower degradation rates were achieved at the lower temperature. As per the results of genomic sequencing, well-known oil degraders metabolized both the dilbit types, but their performance varied. All the enrichments metabolized PBC as well WCS, but the nature and extent of the degradation was distinct. KRE meso culture was the most effective among all, as it completely removed alkanes and PAHs. AFE enrichment performed differently at two temperatures; an acclimation period of 8 days was observed at 5 ºC while there was no lag at 25 °C. KRE meso culture as well as AFE culture at both the temperatures degraded alkanes completely while they were not able to metabolize heavier fractions of the oil (C_{2-4} homologues of 3 ring compounds and 4 ring compounds).

The aquatic effects data generated here for two dilbits to four standard aquatic species indicate that dilbit products can have similar acute and short-term sublethal toxicity as crude oils and other petroleum products, but information is still extremely limited. Acute toxicity of unweathered and weathered dilbit products was similar in all four species, and weathered dilbit products were sublethally toxic at 0.8 to 16 ug/L total petroleum hydrocarbons. These levels are similar to toxicity reported for other aquatic species and oil products. Additionally, for future research, the unique hydrocarbon composition of dilbit consisting of both heavy and light components necessitates including additional analytes such as asphaltenes, C5-C9 alkanes, and additional monoaromatics.

5. References

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